

FOREIGN TRIP REPORT

LEUKEMIA STUDY GROUP  
KIEV, UKRAINE

February 22 - March 7, 1999

ALFRED F. McFEE, Ph.D.  
BIODOSIMETRY

Date Submitted:

March 12, 1999

When I departed Oak Ridge on February 22, 1999, I took with me three vials of chromosome probe material, one unmounted dual bandpass filter set to be installed in the microscope in Kiev, and a variety of tools and reagents that I felt might be necessary for the work there.

- Wednesday, February 24, 1999

I removed an existing filter set from the microscope slider and installed the dual bandpass filter, unpacked and installed the new 100 watt mercury vapor light source on the microscope, and focused and tested the system. It was found to perform very adequately. In the late afternoon, we started to make up the several solutions needed for probing slides and discovered that there is not a working pH meter in the laboratory. Proper pH adjustment of several solutions needed for probing is essential and this poses a serious stumbling block to efficiently carrying out the procedure.

- Thursday, February 25, 1999

Since a pH meter could not be located anywhere in the clinic, our option was to take the various solutions to the laboratory in Puscha where a meter was available and we could perform the necessary adjustments. As a result, what should have been a one-hour job required a full half-day to complete. During these preparations I was struck by the fact that the cytogenetic laboratory does not have adequate laboratory glassware for good quantitative work. They have no volumetric flasks and all measurements are made with graduate cylinders. Solutions are stored in a variety of containers, including soft drink bottles and ordinary jelly jars. One cannot help but be concerned about quality control of critical solutions under such conditions, but we do the best we can and hope. By mid-afternoon we were ready to start the first slides through the FISH probing procedure. The process seemed to go as prescribed and treated slides went into the incubator late in the day to hybridize until Saturday morning.

- Friday, February 26, 1999

Most of the day was spent reviewing with laboratory personnel microscope operation,

examining old slides from their storage (which had been probed many months ago in Oak Ridge), and refreshing the training of personnel in FISH scoring and terminology. Drs. Romanenko and Bazeyka visited for 15-to-20 minutes and I updated them on the status of the microscope and probe procedures. The cytogenetics laboratory has received a new large freezer which has not operated since they received it. It apparently lost its refrigerant during transit and will need some repair. A repairman came the following week and seemed to indicate he could affect the repairs if sufficient money is available.

- Saturday, February 27, 1999

We spent some two hours harvesting cultures from two donors which had been received in the laboratory on Thursday, and preparing slides from these cultures. We discussed a number of possible methods for improving the quantity and quality of metaphases being obtained. Their preparations are not bad considering they are forced to perform whole blood cultures because CO<sub>2</sub> is not available.

We then performed the critical final steps of the first FISH procedure, Eureka!! On this first try, the probed chromosomes were quite bright and very satisfactory for scoring. We opened a bottle of champagne and celebrated.

- Monday, March 1, 1999

I drew up a proposed score sheet for recording translocations and other aberrations found in FISH probed slides and discussed the score sheet at some length with Drs. Pilinskaya and Dybskiy. I had noted, and it surfaced again here, that their scoring experience in other laboratories had involved the diagramming of all observed translocations with some emphasis on identifying cell clones. I tried to impress on them that while this is good science, it is not necessary, nor does it contribute significantly to the utilization of FISH for dosimetry and is very time consuming. We discussed at some length the best methods for scoring slides and for recording data. There seemed to be general agreement and Dr. Dybskiy developed the score sheet in the computer so that it can be printed out.

Olga Svetkova paid a visit to the laboratory in late morning. We updated her on our progress and showed her probed slides. I discussed with her the general status of the program. In the afternoon we made plans for testing the formamide which they have on hand--(they say that it is some six or seven years old)--and performed the first-day procedure for FISH probing of four additional slides.

- Tuesday, March 2, 1999

Had a lengthy discussion with Dr. Pilinskaya on methods for slide scanning to gain the maximum number of metaphases and to insure that none were scored twice. The availability of formamide of adequate quality is a major concern at this time. They have about 1.5 liters of Sigma material, which they say is several years old, but the bottles have not been opened. Another small lot of formamide has been secured from Dr. Bazeyka. We prepared denaturing solutions today using each of these lots of formamide and initiated replicate slides in each. The finished slides will be evaluated on Thursday to test the quality of the formamides. In addition, we initiated the probing of six additional slides. Dr. Pilinskaya began scanning one of the first slides we had probed and over the next two days, we jointly examined and discussed all of the aberrant cells that she encountered. She was ultimately able to score just over 750 metaphases from the slide and this is more than I would have expected. Assuming that this was a representative slide, it means that only two slides will need to be probed from most donors; therefore, the amount of probe they have on hand will be more than adequate to complete the first phase of this study.

Dr. Pilanskaya has expressed concern about their ability to score all the slides during the time remaining in the program. Based on the rate at which she scored this initial slide, with two persons each scoring for the four hours maximum each day, they should be able to complete the scoring in some 65 working days. This is a best case calculation and there will certainly be delays. However, even a factor of 2 should permit scoring to be completed within six months.

- Wednesday, March 3.

We continued to examine aberrant cells with Dr. Pilanskaya and Dr. Dybskiy, as well as Dr. Cherviakova, who also works in the laboratory, and to discuss each aberration in detail. Final processing was performed on the four slides begun on Monday, and each was found to have a very acceptable probe intensity. The first day procedure was performed on six additional slides. Since Monday, I have been strictly an observer of the probe procedure, offering only minor "convenience" suggestions. Dr. Dybskiy has been performing the work and seems quite capable of conducting the procedure.

- Thursday, March 4, 1999

Second day procedures were performed on six slides, four of which were to evaluate the two lots of formamide on hand. The lot of Sigma formamide produced very acceptable results. The other yielded visible probe but of considerably reduced intensity, such that it is deemed unsatisfactory. Since some 1.5 liters of the Sigma formamide is on hand, this should solve the difficult problem of obtaining and shipping formamide.

Drs. Pilanskaya and Dybskiy and I had lunch with Drs. Romanenko and Bazeyka and made a report of our progress. I told Dr. Romanenko that the system was working well and I saw no reason why the chromosome dosimetry portion of the study could not move forward to completion. He seemed very happy with the situation.

- Friday, March 5, 1999

We prepared a one-page written summary of our activities to be forwarded to Dr. Romanenko. The probe procedure was completed on four additional slides. These slides yielded signals which were not quite as bright as in previous days, but still satisfactory. I discussed a number of possible causes for the reduced signal quality and intensity, and procedural modifications that could be made to help overcome them. I also worked with Dr. Dybskiy on methods for preparing slides with a higher concentration of metaphases of adequate quality in order to reduce the time required for scanning in the scoring process.

During the past 10 days we have applied FISH probe to a total of 21 slides, representing 19 donors in the study. All yielded satisfactory results and I would expect the remaining samples to do as well.

### SUMMARY

The microscope for scoring probed slides has been upgraded and is now top of the line and functioning well. Two additional bulbs for the lamp (at about \$150 each) will be needed to complete the more than 600 hours of scoring time anticipated. The FISH probe procedure is working well in the laboratory at this time and Dr. Dibskiy is quite proficient in conducting it. Both Dr. Pilinskaya and Dibskiy have an adequate grasp of procedures and pitfalls associated with the microscopic scoring process and should be able to produce reliable data. I insisted that Dr. Pilinskaya must arrange her activities so as to have half-day blocks of time to be devoted to scoring slides. She tends to let administrative duties and phone calls interfere. I see no reason at this point why the FISH dosimetry portion of the study should not move steadily toward completion. Under good conditions, the 50 samples could be evaluated in about four months, and I see no reason why data should not be gathered and summarized within the next six months.