STATE OF NEW YORK
DEPARTMENT OF HEALTH

Wadsworth Center The Governor Nelson A. Rockefeller Empire State Plaza P.O. Box 509 Albany, New York 12201-0509

Richard F. Daines, M.D.
Commissioner

Molecular Oncology Proficiency Test Program
MODE 6-07
Summary of results[1]

August 27, 2007

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular Oncology from June 25, 2007, MODE 6-07.

Samples: All laboratories received three (3) different specimens prepared by Wadsworth Center personnel. Please note the sample identification prefix of MLIM for the current PT, MODE 6-07, is equivalent to our standard L/L prefix, and the numbers 1-3 correspond regardless of the letter prefix.

Evaluation: Laboratories were asked to perform those molecular assays for which they hold or have applied for a NYS permit. A total of 33 laboratories participated, performing various numbers and combinations of tests. The attached tables summarize the results and methods that were used by participating laboratories. A consensus interpretation of **G**(ermline/normal) or **R**(earranged/positive) is also indicated where possible. Please note that **R** includes anything that is not normal, i.e. that is rearranged, translocated, contains a fusion gene or viral sequences, or contains a mutation. Only truly normal samples, i.e. those without any evidence of a disease-related process of any nature, should be called **G**. **I**(ndeterminate) is shown if no consensus was reached because only one lab performed a test, or if the difference between the number of labs reporting R or G was ≤ 1. However, in cases where there was a clear difference in the results obtained with one method vs. another, e.g. southern blot (SB) vs. PCR, then the "consensus" was expressed for each method separately as e.g. **R/G**, where the first letter belongs to the first method, and the second belongs to the second method. In addition, you also receive a personalized result sheet that gives your lab's result in comparison to the all lab consensus derived from all methods combined. Your score is expressed as a fraction, whereby the denominator is the number of samples you analyzed with a given assay and that were scored, and the numerator is the number of samples for which you agree with the consensus. For example, 3/3 means you analyzed all 3 samples and agreed with the consensus for all 3 of them. 1/2 would mean you analyzed only two samples, and agreed with the consensus for only one of them. If you reported results from two different methods, each method will be scored independently. At this time we did not assign a grade, but may do so in the future. If your result is different from the consensus we ask that you take a careful look at your analysis and investigate why you may have reported a discrepant result. While this may be because of your assay's design or sensitivity and thus does represent an error *per se,* there were also several instances where we suspect either a sample mix-up or a mistake in entering the data on the forms.

[1] The use of brand and/or trade names in this document does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health

NYS#L/L 1:

B-cell tests: For IgH, all 23 laboratories that used PCR reported no rearrangement, as did the two labs that tested for IgH by FISH. In contrast, four out of six labs (67%) that used SB reported a rearrangement; three of these four labs used Dako reagents and one used a home brew method. It is possible that the sample had an IgH rearrangement that could only be detected by SB, though one lab reported that this rearrangement could be a polymorphism. However, we have not further investigated this. All six labs that tested for IgKappa (IgK) by PCR found no rearrangement, as did the three laboratories that tested for IgK by SB. One lab also tested for IgLambda (IgL) by SB, and found no rearrangement. All laboratories that tested for BCL2 t(14;18) by various methods reported no translocation at any of the three breakpoints. Similarly, none of the six laboratories that tested for BCL1 t(11;14) by any method found a rearrangement. Thus, the consensus was that this sample did not exhibit immunoglobulin gene rearrangements, with the exception mentioned above.

T-cell tests: All 23 laboratories that tested for TcRGamma (TcRG) by PCR found a rearrangement. Similarly, all seven labs that tested for TcRBeta by PCR reported a rearrangement. In contrast, four out of seven (57%) labs that tested for TcRBeta by SB reported no rearrangement; three out of these four labs used Dako reagents and one used home brew method. While it is unclear why those four labs missed the TcRβ rearrangement, it is unlikely that it was due to too few tumor cells in the sample. Results from Flow analysis showed between 10-65% malignant cells in the sample. The two laboratories that tested for TcRDelta (TcRD) by SB or PCR found no rearrangement. These results suggest that this sample exhibited T-cell receptor gene rearrangements.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained T-cell receptor gene rearrangements, and the overall diagnosis is a T-cell clone with TcRBeta and TcRGamma rearrangements. This conclusion is in agreement with the result from Flow Cytometry, which indicated an atypical T-cell of an unclear stage of maturity.

NYS#L/L 2:

B-cell tests: For IgH, all 24 laboratories that used PCR reported a rearrangement, as did all six laboratories that used SB. Similarly, all nine labs that tested for IgK (PCR=6, SB=3) reported a rearrangement, whereas the two labs that also tested for IgL by SB or PCR found no rearrangement. No translocations involving bcl-2 or bcl-1 were detected. Thus, these results suggest that this sample contained a B-cell clone with both IgH and IgK gene rearrangements.

T-cell tests: All 22 laboratories that tested for TcRG by PCR found no rearrangement. Similarly, all 14 labs that tested for TcRB by SB and/or PCR, seven for each method, found no rearrangement. Two laboratories also tested for TcRD by SB or PCR, and found no rearrangement. Therefore, there was a general consensus that this sample did not contain a T-cell clone. In contrast to sample L/L 1, there was complete concordance between the results from SB and PCR.

C-myc t(8;14): All four laboratories that tested for t(8;14) by FISH identified the presence of the translocation, which is a marker for Burkitt lymphoma, which indeed was the origin of the malignant cells used in this sample.

P53: Two labs tested for p53, and found a mutation by PCR (I254D) or deletion by FISH, respectively.

IgVH mutation: All three labs that tested for IgVH found mutations in IgVH4-34; two labs reported mutations in 2% of alleles, and one lab reported IgVH4-34 hypermutated.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a B cell clone with c-myc translocation, and possible P53 and IgVH mutations. The overall diagnosis is consistent with the results from Flow Cytometry, which indicated an intermediate B-cell.

NYS#L/L 3:

B-cell tests: For IgH, all 23 laboratories that used PCR reported no rearrangement, as did all six laboratories that used SB and the two labs that used FISH. All nine labs that tested for IgK (PCR=6, SB=3) reported no rearrangement, as did the two labs that also tested for IgL by SB or PCR. No translocations involving bcl-2 or bcl-1 were detected. Thus, these results suggest that this sample did not contain a B-cell clone.

T-cell tests: 20 out of the 22 laboratories (91%) that tested for TcRG by PCR found no rearrangement. Of the two labs that did not agree, one found a weak rearrangement signal by primer Vγ9, and one was indeterminate due to a small peak above the polyclonal background. Similarly, all 14 labs that tested for TcRB by SB and/or PCR, seven for each method, found no rearrangement. Two laboratories also tested for TcRD by SB or PCR, but found no rearrangement. Therefore, there was a general consensus that this sample did not contain a T-cell clone.

MLL: Three labs (75%) tested for MLL(11q)-associated translocations by FISH, and found a translocation, whereas the two labs that used a home brew RT-PCR method reported no translocation; however, both only tested for MLL-AF4, which suggested that the translocation involved one of the other two potential partners on chromosome 9 or 19, respectively.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a MLL translocation. This is consistent with the result from Flow Cytometry, which indicated a myelomonocytic cell at an aberrant monocytic stage of differentiation.

In summary, there was good agreement between the molecular and the flow cytometry results. However, the analysis by molecular methods clearly provided additional information by finding that sample L/L 2 contained the c-myc t(8;14) translocation and possibly P53 and IgVH mutations, and L/L 3 contained a MLL translocation. Thus, these two approaches are complementary rather than redundant. In this PT, we asked for an overall diagnosis for each sample, and required you to provide this information as in the patient test report. In general, laboratories responded well and the answers indicated a general diagnostic consensus, although the actual wording varied from lab to lab. While we recognize that PT reporting is somewhat artificial, we still urge you to make sure that your interpretation matches the analytical results.

The attached tables show a summary of the results, and figure 1 shows the DNA and RNA yield distributions for the three samples. RNA yields from samples L/L1, 2, and 3 ranged from a minimum of 3.4, 2.9, and 3.0 µg to a maximum of 38.4, 60, and 41 µg, respectively, corresponding to an about 11- to 20-fold difference between lowest and highest yield for each sample. DNA yields for samples L/L1, 2 and 3 also ranged broadly from 4.0, 2.9, and 2.3 µg to 550, 540, and 424 µg, respectively, corresponding to an about 137- to 186-fold difference between lowest and highest yield for each sample. Presumably, the

methods used for DNA and RNA isolation contributed to this wide range. However, it also raises the question of how accurate some of the measurements are. We realize that shipping the samples at room temperature is suboptimal for subsequent RNA analysis. However, because of the combined shipping with the malignant immunophenotyping samples we cannot change that.

Finally, some general comments. There seems to be some confusion as to where to write your results. **Please note: RT stands for reverse transcription, not real time, and thus should only be used for assays whose starting material is RNA. If your starting material is DNA you must record your result in the PCR column.** There were four labs that entered their bcr/abl results in the PCR column. Please make sure that your results are written in the correct column that corresponds to the method you used. Also, there were a few labs that did not indicate the methods and/or reagents that they used for their assays. We cannot properly evaluate your results without this information. Furthermore, contrary to earlier instructions, we now request that you analyze the samples by all molecular tests performed in your lab for which you hold or have applied for a NYS permit.

If you have any questions, comments or suggestions, you may contact me by phone or email at 518-474-2088 or schneid@wadsworth.org. For specific questions about your lab's report please contact Ms. Susanne McHale at (518)486-5775 or smchale@wadsworth.org.

The dates for the Molecular Oncology PT mail-out in 2007 are:

Mail-out date	**Due Date**
October 29	November 26

Sincerely,

Erasmus Schneider, Ph.D.
Director, Oncology Section
Clinical Laboratory Evaluation Program
Wadsworth Center, Room E604
Empire State Plaza
Albany, NY 12201-0509