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

Wendy E. Saunders
Chief of Staff

Molecular and Cellular Tumor Marker Proficiency Test Program
MCTM 10-07
Summary of results[1]

December 26, 2007

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Marker from October 29, 2007, MCTM 10-07.

Samples: All laboratories received three (3) different specimens prepared by Wadsworth Center personnel.

Evaluation: Laboratories were asked to perform those molecular assays for which they hold or have applied for a NYS permit. A total of 35 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** (Germline/normal) or **R** (Rearranged/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** (Indeterminate) 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. Two scores were calculated, one for each assay (**assay score**) across all three samples, and one for each sample (**sample score**) across all assays performed by your lab for each sample. From the latter we also calculated an overall score. Your **assay** 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

[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

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 was scored independently. This score is indicated in the 'score' column to the right of each assay you performed. The **sample** score was calculated as the percentage of 'correct' answers per sample (i.e. that agree with the consensus), based on the number of assays performed per sample that were evaluable. Assays for which no clear consensus was obtained, as indicated by "I", were not included in the score calculation. At the bottom of each sample column on your result sheet you will find the number of assays performed by your lab for the sample, the number of results that were evaluable and used to calculate the score, and the number of 'correct' answers. The actual sample score as % 'correct' answers was calculated by dividing the number of 'correct' answers by the number of evaluable answers times 100. Finally, we also calculated an overall sample score as the average of the three individual sample scores. At this time we did not assign a grade, but may do so in the future. If any of your results are different from the corresponding 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 and/or sensitivity and thus does not represent an error *per se,* it could also be a true error, indicate suboptimal performance of your assay, or be due to a contamination.

NYS#L/L 1:

B-cell tests: For IGH, 27 out of 28 (96%) laboratories that used PCR reported no rearrangement; the one lab that detected a rearrangement used a home brew method. For the other 27 labs, the use of a home brew method versus using commercial primers was evenly split, with 14 labs using a homebrew, and 13 labs using either the Biomed-2 (8) or the original IVS (5) primers. In contrast to the PCR results, all five labs that used SB reported a rearrangement; three of these five labs used Dako reagents and two used a home brew method. It is possible that the sample had an IGH rearrangement that could only be detected by SB due to the lack of proper PCR primers. Two labs tested IGH by FISH; one detected a rearrangement, the other detected none. All seven 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 PCR, and found no rearrangement. All laboratories that tested for IGH/BCL2 by various methods reported no translocation at any of the three breakpoint clusters. Similarly, none of the seven laboratories that tested for the IGH/CCND1 translocation (also known as Bcl-1) by any method found a rearrangement. Thus, the consensus was that this sample contained cells with an IGH gene rearrangement that was only detected by SB.

T-cell tests: All 24 laboratories that tested for TcRGamma (TRG) by PCR found a rearrangement. Similarly, six out of seven (86%) labs that tested for TcRBeta (TRB) by PCR reported a rearrangement. In contrast, four out of seven (57%) labs that tested for TRB by SB reported no rearrangement; two out of these four labs used Dako reagents, one used a home brew method, and one did not indicate what method was used. While it is unclear why those four labs missed the TRB rearrangement, it is unlikely that it was due to too few tumor cells in the sample. Results from Flow analysis showed 35-54% malignant cells in the sample. The one laboratory that tested for TcRDelta (TRD) by SB and PCR found no rearrangement. These results suggest that this sample contained cells with T-cell receptor gene rearrangements.

P53: One lab out of two (50%) detected a nonsense mutation (292C>T) by PCR, whereas no chromosomal rearrangement was detected at this locus by two labs by FISH.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal T-cell population of cells with possibly also an IGH gene rearrangement. This conclusion is in agreement with the result from Flow Cytometry, which indicated an immature thymocyte.

NYS#L/L 2:
B-cell tests: For IGH, all 28 laboratories that used PCR reported a rearrangement, as did all six laboratories that used SB. Similarly, of the ten labs that tested for IGK (PCR=7, SB=3), nine reported a rearrangement. Interestingly, one lab tested IGK using either home brew or IVS primers and the results were not consistent: no rearrangement was detected by the home brew method, whereas a rearrangement was detected by the IVS primers. This lab needs to further evaluate their home brew primers. The one lab that also tested for IGL by SB and PCR found no rearrangement. No translocations involving IGH/BCL2 or IGH/CCND1 (Bcl-1) were detected. Thus, these results suggest that this sample contained a B-cell clone with both IGH and IGK gene rearrangements. In contrast to sample L/L 1, there was complete concordance between the results from SB and PCR.

T-cell tests: Fourteen out of 24 laboratories (58%) that tested for TRG by PCR found no rearrangement, whereas eight found a rearrangement and two labs reported an indeterminate result. Among the eight labs that found a rearrangement, three used the Biomed-2 primers (two found **R** with tube B primers, one with both tube A and B primers), three used home brew primers (two found **R** with Vγ1-8 primers, one used mixed primers that did not differentiate individual segments), one used the original IVS primers and found **R** with both primer mixes 1 and 2, and one did not indicate what primers were used. Thus, there was no clear pattern of primers used that could explain the discrepancy in results. Five out seven (71%) labs that tested for TRB by PCR found no rearrangement, as did all seven labs that used SB. The two labs (29%) that reported a TRB rearrangement by PCR used the Biomed-2 tube C primers and reported a rearrangement for TRB in all three samples; they also reported a rearrangement for TRG in all three samples, with one using the Biomed-2 tube A and B primers, and the other using home brew Vγ1-8 primers. These labs should check for a possible contamination in their assays. One laboratory also tested for TRD by SB and PCR, and found no rearrangement. Thus, there was a majority consensus that this sample did not contain cells with T-cell receptor rearrangements, although a substantial number of labs disagreed.

EBV: All four laboratories identified the presence of EBV by PCR, which is usually associated with B-cell neoplasms.

IGHV mutation: Five labs tested for IGHV hypermutation, and all concluded that this sample belonged to the IGHV3-13 family, but was not hypermutated. Please note, there was some confusion on how to report the results, with some labs reporting “R” despite reporting the result as unmutated. Since hypermutation was not detected we replaced all Rs with Gs in our summary table. We will make some changes to the result form that hopefully will make reporting for this test clearer in the future.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a B-cell clone with presence of EBV. The overall diagnosis is consistent with the results from Flow Cytometry, which indicated an early B cell.

NYS#L/L 3:

B-cell tests: For IGH, all 27 laboratories that used PCR reported no rearrangement, as did all six laboratories that used SB and the two labs that used FISH. All ten labs that tested for IGK (PCR=7, SB=3) reported no rearrangement, as did the one lab that also tested for IGL by SB and PCR. Twelve out of thirteen labs (92%) did not detect an IGH/BCL2 translocation and no translocations involving IGH/CCND1 were detected. Thus, these results suggest that this sample did not contain a B-cell clone.

T-cell tests: 20 out of the 24 laboratories (83%) that tested for TRG by PCR found no rearrangement. Of the four labs that did not agree, three used a home brew method (one found **R** with primers Vγ9 and 11, another with Vγ1-8; one used mixed primers that did not differentiate individual segments) and one found **R** with the Biomed-2 primers (both tube A and B primer mixes); three of these four labs also reported a TRG rearrangement for the L/L 2 sample, which disagreed with the majority consensus. Five out of seven (71%) labs that tested for TRB by PCR found no rearrangement as did the seven labs that used SB. The two labs (29%) that reported a TRB rearrangement by PCR used the Biomed-2 tube C primers and reported a rearrangement for TRB in all three samples; they also reported a rearrangement for TRG in all three samples, with one using the Biomed-2 tube A and B primers, and the other using home brew Vγ9 and 11 primers. These labs should check for a possible contamination in their assays. One laboratory also tested for TRD by SB and PCR, but found no rearrangement. Therefore, there was a general consensus that this sample did not contain a T-cell clone, although a few labs disagreed.

BCR/ABL1: All 19 labs that tested for BCR/ABL1 MBR translocations by RT-PCR found a translocation, as did three out of the four labs (75%) that gave their method as PCR. All six labs that did not distinguish MBR from mcr by RT-PCR detected a translocation, as did four of the five labs (80%) that used FISH. Interestingly, eight out of sixteen labs (50%) also detected a BCR/ABL1 mcr translocation by RT-PCR (seven) or PCR (one), although at a much lower level than the BCR/ABL1 MBR translocation (see table below).

	Lab 1[1]	Lab 2	Lab 3	Lab 4
MBR	1.06(bcr-abl/abl)	0.999(bcr-abl/abl)	127(% positivity)	3.1
mcr	0.0042	0.0001	0.081	R(Qual)
MBR/mcr	252	9990	1567	

[1]Only labs that gave quantitative results are included.

Therefore, there was a general consensus that this sample contained the BCR/ABL1 p210 fusion gene, but there was no consensus as to the presence of the BCR/ABL1 p190 fusion transcript. The attached table and figure 2 show the BCR/ABL1 results from those fifteen laboratories that performed the assay quantitatively. Five different housekeeping genes were used for normalization, and the results were expressed either as a ratio of bcr/abl copies to housekeeping gene copies, or as a percentage of the mean

diagnostic CML. The results varied considerably, reflecting the use of different housekeeping genes. Furthermore, even quantitative values that were normalized to the same housekeeping gene, e.g. abl, ranged from 25.82%, or a ratio of 0.26 to a ratio of 24.17, representing a 96-fold difference. It is possible that the lab reporting a ratio of 24.17 really meant 24.17%, but this was not indicated. Clearly, further standardization is required if the results are to become comparable between different laboratories.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a BCR/ABL1 translocation. The result from Flow Cytometry indicated a possible promyelocyte/myelocyte.

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 EBV, and L/L 3 contained a BCR/ABL1 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 0.5, 1.3, and 0.8µg to a maximum of 42.9, 117.5, and 72 µg, respectively, corresponding to an about 80-fold difference between lowest and highest yield for each sample. DNA yields for samples L/L1, 2 and 3 also ranged broadly from 6.7, 8, and 6 µg to 290, 760, and 192 µg, respectively, corresponding to an about 32- to 95-fold difference between lowest and highest yield for each sample. One lab reported 760 µg for L/L 2 DNA yield, which is 493 µg more than the next highest DNA yield that was 267.5 µg. 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. We asked labs to report the results as R, G or I as described previously. Two labs entered the EBV results as numeral data only, without providing an interpretation of these numbers. When performing a quantitative test and reporting the quantitative result we ask that you also provide an interpretation of these results. For example, is a result given as <100 significant and would thus be qualified as "R", or is such a result, which presumably is below the limit of quantification (LOQ), considered normal and then should be "G". Furthermore, there still 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. Vice versa, if your starting material is RNA, you must report your results in the RT-PCR column. For example, there were four labs that entered their BCR/ABL1 results in the PCR column; unless they used genomic DNA as their starting material, the results should have been written in the RT-PCR column. Thus, please make sure that your results are written in the correct column that corresponds to the method you used. Also, seven labs did not indicate their units for the BCR/ABL1 result for L/L 3 as required, although a quantitative method was used. Note: every quantitative result must

be accompanied by the appropriate unit. A few labs did not indicate the methods and/or reagents that they used for their assays. We cannot properly evaluate your results without this information. Finally, we ask 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 or the evaluation please contact Ms. Susanne McHale at (518) 486-5775 or smchale@wadsworth.org, or Dr. Rong Yao at (518) 474-1744 or yaor@wadsworth.org.

The dates for the Molecular and Cellular Tumor Marker PT mail-outs in 2008 are:

Mail-out date	**Due Date**
February 25	March 25
June 23	July 22
October 27	November 25

Sincerely,

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