



STATE OF NEW YORK DEPARTMENT OF HEALTH

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Molecular and Cellular Tumor Marker Proficiency Test Program MCTM 6-08 Summary of results¹

August 22, 2008

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from June 23, 2008, MCTM 6-08.

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 34 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. 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 evaluable, 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

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reported results from two different methods, each method was scored independently, and the results added together. 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 either the assay or sample 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, indicating suboptimal performance of your assay, or be due to a contamination.

NYS#L/L 1:

B-cell tests: For IGH, all 32 laboratories reported no rearrangement (26= PCR, 4= SB, 2= FISH). Five out of six labs (83%) that tested for IgKappa (IGK) by PCR found no rearrangement, as did the two laboratories that tested for IGK by SB. The two labs that also tested for IgLambda (IGL) found no rearrangement by SB and PCR, respectively. All laboratories that tested for IGH/BCL2 by various methods reported no translocation at any of the three breakpoint clusters. Similarly, none of the eleven 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 did not contain immunoglobulin gene rearrangements.

T-cell tests: Eighteen out of 26 (69%) laboratories that tested for TcRGamma (TRG) by PCR found a rearrangement, possibly in the V γ If, V γ 10, and V γ 1-8 regions that were detected by either Biomed-2 tube A or home brew V γ 1-8 primers (Table 6). The other eight labs that either used home brew primers V1-8, 9, and 11 (five labs), or the IVS mixes 1 and 2 (three labs) reported no rearrangement, which was against the majority consensus, possibly due to different primer designs. All twelve labs that tested for TcRBeta (TRB) reported a rearrangement (4=SB, 8=PCR), possibly in the V β and J β 2 region detected by the Biomed-2 tube B (Table 7). The two laboratories that tested for TcRDelta (TRD) found no rearrangement (1=SB, 1=PCR). These results suggest that this sample contained cells with T-cell receptor beta and gamma gene rearrangements.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal T-cell population. This conclusion is in agreement with the result from Flow Cytometry, which indicated a common thymocyte with an aberrant expression of CD34.

NYS#L/L 2:

B-cell tests: For IGH, all 26 laboratories that used PCR reported no rearrangement. Similarly, two out of three (67%) labs that used SB and one out of two labs that used FISH reported no rearrangement. In contrast, all seven labs that tested for IgKappa (IGK) by PCR found a rearrangement, possibly in the Vk,

Jk, and Kde regions detected by both Biomed-2 A and B tubes (Table 5); however, the one laboratory that tested for IGK by SB reported no rearrangement. One lab also tested for IgLambda (IGL) by SB, 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 ten 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 immunoglobulin kappa gene rearrangement.

T-cell tests: 25 out of 26 laboratories (96%) that tested for TcRGamma (TRG) by PCR found no rearrangement. Likewise, all twelve labs that tested for TcRBeta (TRB) reported no rearrangement (4=SB, 8=PCR). The two laboratories that tested for TcRDelta (TRD) also found no rearrangement (1=SB, 1=PCR). These results suggest that this sample did not contain cells with T-cell receptor gene rearrangements.

EBV: All four labs detected the presence of EBV virus sequences by PCR, which is usually associated with Burkitt lymphoma.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal B-cell population. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of an aberrant B cell population, although this sample had an inadequate number of aberrant cells to allow for a definitive immunophenotype to be assigned.

NYS#L/L 3:

B-cell tests: For IGH, all 26 laboratories that used PCR reported a rearrangement, as did all four laboratories that used SB and the two labs that used FISH. Rearrangements were detected by the Biomed-2 tubes A and B that target the FR1 and FR2 regions, but not tube C that targets FR3 (Table 4). Interestingly, however, rearrangements were detected in all three frameworks by the IVS and home brew primers (Table 4), possibly because of different (less FR-specific?) primer design. Similarly, all eight labs that tested for IGK (PCR=6, SB=2) reported a rearrangement, possibly in the Kde region detected by the Biomed-2 tube B (four labs, Table 5). The two labs that also tested for IGL found no rearrangement (1=SB, 1=PCR). No translocations involving IGH/BCL2 MBR, mcr, and MBR3' by SB and PCR, or IGH/CCND1 (Bcl-1) were detected by any methods. However, an IGH/BCL-2 was reported by three labs that used FISH. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements and an IGH/BCL-2 translocation.

T-cell tests: 20 out of 26 laboratories (78%) that tested for TRG by PCR found no rearrangement, whereas five labs reported a rearrangement and one was indeterminate. Seven out of eight (88%) labs that tested for TRB by PCR also indicated no rearrangement, as did all four labs that used SB. Similarly, the two labs that tested for TRD found no rearrangement (1=SB, 1=PCR). These results suggest that this sample did either not contain cells with T-cell receptor gene rearrangement, or only a very minor population of cells.

IGHV mutation: Six labs tested for IGHV hypermutation. Of those, four labs reported IGHV hypermutation; three detected between 8.0-8.5% mutation in the IGHV3-48 family, whereas one lab

assigned the clonal IGHV region to the 5-51 family, but did not indicate the mutation rate. The two other labs that performed this test failed to detect a clonal band, suggesting a problem with their PCR.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a B-cell clone with IGHV hypermutation and IGH/BCL-2 translocation. The overall diagnosis is consistent with the results from Flow Cytometry, which indicated an early B cell.

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 EBV sequences, and that L/L 3 contained an IGH/BCL-2 translocation and IGHV hypermutation. Furthermore, because of the low number of malignant cells in sample L/L 2, immunophenotyping was inconclusive, whereas the molecular analyses were clearly able to identify this sample as a B-cell clone. 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 though a few labs did not provide this information. 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 both in aggregate (Tables 1-3) as well as by individual primer mixes for the B- and T-cell tests (Tables 4-7). Figure 1 shows the DNA and RNA yield distributions for the three samples. DNA yields from samples L/L1, 2, and 3 ranged from a minimum of 9.8, 3.1, and 7.4 μg per 5 ml specimen to a maximum of 5004, 1004, and 4581 μg , respectively, corresponding to an about 619- to 323-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 14, 5, and 17 μg to 2665, 2224, and 4008 μg , respectively, corresponding to an about 444- to 190-fold difference between lowest and highest yield for each sample. Please make sure that you report the DNA/RNA yields calculated for the entire 5 ml sample even if you only extract it from a much smaller volume. One lab reported 2665 μg for L/L 1 RNA yield, which is almost twice the next highest RNA yield that was 1407 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. 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. Please make sure that your results are written in the correct column that corresponds to the starting material you used. Some labs that used FISH only entered their results as the percentage, e.g. 76%; those labs should report their result as R or G along with the percentage of cells with translocation or deletion so that we are able to score the results. 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 date for the next Molecular and Cellular Tumor Marker PT mail-out in 2008 is:

Mail-out date
October 27

Due Date
November 25

Sincerely,

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