STATE OF NEW YORK
DEPARTMENT OF HEALTH

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Molecular and Cellular Tumor Marker Proficiency Test Program
MCTM 2-09
Summary of results[1]

April 22, 2009

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from February 23, 2009, MCTM 2-09.

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** (but see discussion below for TRB for L/L 1). **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 is not sufficient to derive a clear consensus. 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 reported results from two different methods, each method was scored independently, and the results added

[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

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 (Table 1):
B-cell tests: For IGH, 22 out of 28 labs (79%) reported that this locus was rearranged by PCR. Four out of six labs (67%) that reported G used home brew primers for FR2 and FR3 only, and possibly missed a rearrangement in the frame work 1, whereas all five labs using the original IVS FR1 primers and seven out of eight (88%) labs using the Biomed-2 tube A detected this rearrangement (Table 4). Another interesting fact was that seven out of nine labs (78%) using the Biomed-2 tube B that detects the FR2 rearrangement reported a rearrangement; however, only two out of five labs (40%) using the IVS FR2 and one out of ten labs (10%) using home brew FR2 primers reported this rearrangement. One of the possible reasons could be that the Biomed-2 tube B contains additional primer pairs that detect this rearrangement in the frame work 2 compared to the IVS or home brew primers. Another interesting observation was that the majority (62%) of Biomed-2 tube C results were G, whereas the majority of IVS (83%) or home brew primers (71%) for FR3 did detect a rearrangement. Thus, while the results for individual primer mixes are somewhat inconsistent, overall there was still a consensus that this sample contained cells with a IGH rearrangement. This conclusion is further supported by the results from Southern blot, with all three labs also reporting a rearrangement, as well as by FISH. Similarly, ten out of eleven labs (91%) that tested for IGK reported a rearrangement using either primers B by the Biomed-2 or home brew primers that target Vk and Jk regions (Table 5); the two labs that used SB also detected a rearrangement. Two labs that tested for IGL (SB=1, PCR=1) detected no arrangement. No translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any methods. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: 25 out of 28 laboratories (89%) that tested for TRG by PCR found no rearrangement. In contrast seven out of nine (78%) labs that tested for TRB by PCR reported a rearrangement, of which six used the Biomed-tube C (Table 7). Since the tube C possibly detects an incomplete (D-J) TCR beta gene rearrangement that usually has a poor correlation with the neoplastic process in the T lineage, the significance of this result is unclear. However, two out of four labs also detected a rearrangement by SB (one used Dako and another used HB TRB probes). Thus, while a majority of labs detected a possible TRB gene rearrangement analytically, its clinical significance is unclear. The two laboratories that tested for TcRDelta (SB=1, PCR=1) also found no rearrangement.

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

IGHV mutation: Three out of eight labs (38%) that tested reported IGHV hypermutation (PCR=2, RT-PCR=1), of which two assigned it to the IGHV3-9 family, and reported mutation rates between 5.3-6.3%. The third lab indicated the mutation belonged to the VH2-5 family, but did not specify the mutation rate. In contrast, the other five labs did not even detect a clonal band.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal B-cell population possibly with IGVH hypermutation. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of an early B cell population.

NYS#L/L 2 (Table 2):
B-cell tests: For IGH, all 34 laboratories reported no rearrangement (PCR=28, SB=3, FISH=3). Likewise, all thirteen labs reported no rearrangement for IGK (PCR=11, SB=2), as did two labs for IGL (PCR=1, SB=1). All laboratories that tested for IGH/BCL2 by various methods reported no translocation at any of the three breakpoint clusters. Similarly, none of the nine laboratories that tested for the IGH/CCND1 translocation (also known as Bcl-1) by any method found a rearrangement. Thus, the overall consensus was that this sample did not contain any immunoglobulin gene rearrangements.

T-cell tests: all 28 laboratories that tested for TcRGamma (TRG) by PCR found a rearrangement, possibly in the Vγ1-8 and 10 regions (Table 6). Eight out of nine (89%) labs that tested for TcRBeta (TRB) reported a rearrangement by PCR, possibly in the Vβ and Jβ regions detected by the Biomed-2 tube A primers (Table 7). The one lab that did not detect TRB arrangement used home brew primers. All four labs that tested TRB by SB reported a rearrangement, supporting the PCR results. The two laboratories that tested for TcRDelta (TRD) found no rearrangement (PCR=1, SB=1). These results suggest that this sample contained cells with T-cell receptor gamma and beta 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 promothymocyte or immature T cell population.

NYS#L/L 3 (Table 3):
B-cell tests: For IGH, 27 out of 28 laboratories (96%) that used PCR reported a rearrangement, which was confirmed by all three results from SB. Rearrangements were detected by all labs that used the Biomed-2 tubes A, B, or C that target all three framework regions (Table 4). Interestingly, all fourteen labs reported a rearrangement with the home brew FR3 primers, as did the five out of six labs (83%) that used the IVS FR3 primers. However, majority of labs that used the IVS (60%) or HB (70%) FR1 and FR2 primers detected no rearrangement. Similar to the FR2 results in L/L 1 one of the possible reasons could be that the Biomed-2 IGH Tubes A and B contain additional primer pairs that detect rearrangements in these frame works that are not detected by the IVS or HB primers. All thirteen labs that tested for IGK (PCR=11, SB=2) reported a rearrangement, whereas the two labs that also tested for IGL (SB=1, PCR=1) found no rearrangement. No translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any methods. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: 27 out of 28 laboratories (96%) that tested for TcRGamma (TRG) by PCR found no rearrangement. Likewise, eight out of nine labs (89%) that tested for TcRBeta (TRB) reported no rearrangement, as did all four labs that used SB (Table 3). The one lab that reported rearrangements using the Biomed-2 TRG and TRB primers is the same lab that did not detect the IGH rearrangement, raising the question of a primer or sample mix-up (Tables 6 and 7). Two labs that tested for TRD found no rearrangement by SB and PCR, respectively. These results suggest that this sample did not contain cells with T-cell receptor gene rearrangement.

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

IGHV mutation: Four out of eight labs (50%) that tested reported IGHV hypermutation (PCR=2, RT-PCR=2), of which three assigned it to the IGHV4-31 family, and reported mutation rates between 8.3-9.7%. The fourth lab indicated the mutation belonging to the VH2-5 family, but did not specify the mutation rate. In contrast, the other four labs did not even detect a clonal band.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a B-cell clone possibly with IGHV hypermutation. The overall conclusion is consistent with the results from Flow Cytometry, which indicated an early B cell population.

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 1 and 3 contained EBV sequences, and possible IGHV hypermutation. 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, there was a good consensus in the diagnoses given. 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 5.0, 0.5, and 5.0 µg per 5 ml specimen to a maximum of 8388, 7548, and 10176 µg, respectively, corresponding to a 1677- to 15096-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 3.5, 4, and 3.5 µg to 3375, 2437.5, and 1937.5 µg, respectively, corresponding to a 553- to 964-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 smaller aliquot. 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 **r**everse **t**ranscription, 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 a percentage, e.g. 76%; those labs should indicate whether their interpretation of this result is R or G. 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. A couple of labs did not indicate their overall diagnosis for three PT samples, please be sure to include this information so we can evaluate your results properly. 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-out in 2009 are:

Mail-out date	**Due Date**
June 22	July 21*
October 26	November 24*

* = corrected date

Sincerely,

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