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DEPARTMENT OF HEALTH

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Molecular and Cellular Tumor Marker Proficiency Test Program
MCTM 6-09
Summary of results[1]

August 31, 2009

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from June 22, 2009, MCTM 6-09.

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 37 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 was judged 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

[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

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 (Table 1):
B-cell tests: For IGH, 28 out of 29 (97%) laboratories that used PCR reported no rearrangement, as did all three laboratories that used SB and the two labs that used FISH. Twelve labs that tested for IGK (PCR=10, SB=2) reported no rearrangement, as did the one lab that also tested for IGL by SB and PCR. Only one lab reported a rearrangement for IGK by PCR using the Biomed-2 tube A primers. Fifteen labs tested for but 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: All 29 laboratories that tested for TRG by PCR found no rearrangement. Similarly, all nine labs that tested for TRB by PCR found no rearrangement as did the four labs that used SB. 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.

BCR/ABL1: All 28 labs that tested for BCR/ABL1 MBR translocations by RT-PCR found a translocation, as did one lab that used SB. Six out of seven labs that did not distinguish MBR from mcr by RT-PCR also detected a translocation, as did all three labs that used FISH. Interestingly, twelve out of twenty four labs (50%) also detected a BCR/ABL1 mcr translocation by RT-PCR, although at a much lower level than the BCR/ABL1 MBR translocation (Figure 2).

The results from all other tests performed were negative.

Therefore, there was a general consensus that this sample contained cells that express the BCR/ABL1 p210 fusion gene, but there was no consensus as to the presence of the BCR/ABL1 p190 fusion transcript. Figure 2 and the associated table show the BCR/ABL1 results from those 26 laboratories that performed the assay quantitatively. Five different housekeeping genes were used for normalization, and the results were either expressed as a ratio of bcr/abl copies to housekeeping gene copies or as a percentage. 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 13.29%, or a ratio of 0.13 to a ratio of 9.1, representing a 70-fold difference (though it is possible that this particular lab really is expressing its result as %, but erroneously reported as a ratio). The same caveat may also apply to some of the other results, especially in the G6PDH column. Thus, we strongly urge the labs to pay careful attention to the units they report.

These results indicate that the sample contained cells with the t(9;22) translocation expressing the p210 BCR/ABL1 fusion gene. The result from Flow Cytometry indicated a myeloid phenotype. Together, the results are consistent with CML.

NYS#L/L 2 (Table 2):
B-cell tests: For IGH, all 29 labs that used PCR reported a rearrangement with frame work 2 primers (Table 4). One interesting observation was that seven out of eight labs (88%) using the Biomed-2 tube C that detects the FR3 rearrangement reported no rearrangement, whereas all five labs using the IVS FR3 and all thirteen labs using home brew FR3 primers reported this rearrangement. One of the possible reasons could be that the Biomed-2 tube C does not contain the primer pairs that detect this rearrangement in the frame work 3 compared to the IVS or home brew primers. Thus, while there was a difference in the results for individual primer mixes, overall there was still a 100% consensus that this sample contained cells with an IGH rearrangement. In contrast, there was no consensus for IGH by either SB or FISH; two out of three labs (67%) that used SB detected no rearrangement using Dako and home brew probes, respectively, whereas one out of two labs detected a rearrangement by FISH. For IGK, ten out of eleven labs (91%) reported a rearrangement using either the Biomed-2 tube A or home brew primers that target Vk and Jk regions (Table 5). There was no consensus for IGK by SB as one of two labs detected a rearrangement whereas the other did not. The one lab that also tested for IGL by SB and PCR detected no arrangement. No translocations involving IGH/BCL2 MBR (except for one lab that used home brew primers), mcr, and MBR3', or IGH/CCND1 (Bcl-1, 7/8 labs) 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: 19 out of 29 laboratories (66%) that tested for TRG by PCR found a rearrangement. All six labs that used the Biomed-2 tube A primers targeting the Vγ10 region reported a rearrangement, whereas four out of five labs (80%) that used home brew Vγ10 primers reported no rearrangement. Conversely, six out of seven labs (86%) that used home brew Vγ11 primers reported a rearrangement, but all six labs that used the Biomed-2 tube B primers targeting the Vγ9+11 regions reported no rearrangement. Ten out of 29 labs (34%) did not detect any TRG rearrangements. Because of the discrepancies of the results we decided to classify this sample as I (Indeterminate) for TRG and thus non-evaluable, and not to use the consensus to score the labs. For TRB eight out of nine (89%) labs that tested by PCR reported a rearrangement, of which six used the Biomed-tube C (Table 7). Since 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 home brew TRB probes). Thus, a majority of labs detected possible TRG and/or TRB gene rearrangements analytically; however, their clinical significance is unclear, and several labs commented to this effect. The one lab that also tested for TRD by SB and PCR detected no arrangement.

IGHV hypermutation: Seven out of nine labs (78%) that tested reported IGHV hypermutation (PCR=3, RT-PCR=4), of which six assigned it to the IGHV3-7 family, and four reported mutation rates between 8-8.3%, whereas the other two did not specify the mutation rate. One lab indicated that the variable region belonged to the IGHV5-51 family with a mutation rate of 9.46%.

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 IGHV hypermutation. Though the clinical significance of the TRG and /or TRB rearrangements is unclear, the results are reminiscent of a case of B-CLL described by Perl et al. that exhibited both TRG and TRB rearrangements in conjunction with IGH and IGK rearrangements without evidence of a separate

T-cell clone (Perl A. et al. 1990, Leukemia Research 14:131-137). This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of a clonal B cell population, but not a separate distinct T-cell population.

NYS#L/L 3 (Table 3):
B-cell tests: For IGH, there was unanimous agreement that this gene was rearranged (29=PCR, 3=SB, 2=FISH). Rearrangements were detected by PCR using the Biomed-2 tubes A to D that target all three framework regions and also six DH regions, except with the tube E that targets only the DH 7 region. Similar results were obtained by the labs that used either the original IVS or home brew primers (Table 4). Similarly, all thirteen labs that tested for IGK (PCR=11, SB=2) reported a rearrangement, as did the one lab that also tested for IGL by SB and PCR. Either Biomed-2 tubes or home brew A and B detected the rearrangement (Table 5). With one exception, no lab reported a translocation involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1). Thus, these results suggest that this sample contained a B-cell clone with IGH, IGK, and possibly IGL gene rearrangements.

T-cell tests: All 29 laboratories that tested for TRG by PCR found no rearrangement. Likewise, all thirteen labs that tested for TRB reported no rearrangement (4=SB, 9=PCR, Table 7). The one laboratory that tested for TRD by SB and PCR also found no rearrangement. These results suggest that this sample did not contain cells with T-cell receptor gene rearrangements.

MYC t(8;14): All three labs that used FISH identified the presence of the translocation, which is a marker for Burkitt lymphoma.

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

P53: Two labs detected the M237I mutation in exon 7 of the P53 gene by PCR and sequencing; in contrast the three labs that used FISH reported no alteration of the P53 locus on chromosome 17.

IGHV mutation: Eight out of nine labs (89%) reported IGHV hypermutation (3=PCR, 5=RT-PCR), of which seven assigned it to the IGHV3-7 family, and four reported mutation rates between 7.19-7.3%. One lab assigned it to the family IGHV1-3 but did not give the mutation rate.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal B-cell population with presence of EBV, MYC gene translocation, and IGVH hypermutation. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of clonal 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 2 and 3 contained IGHV hypermutation and L/L 3 contained EBV sequences, a P53 mutation, and MYC 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, 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 0.7, 0.7, and 1.0 µg per 5 ml specimen to a maximum of 4572, 1414, and 2646 µg, respectively, corresponding to a 2020- to 6531-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 13, 2.6, and 10.8 µg to 3171, 581, and 1992 µg, respectively, corresponding to a 184- to 243-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. A few labs did not indicate the methods and/or reagents that they used for their assays. Also, if you do buy and use the TRG kit from the InVivoScribe, do not list your method or primers as home brew, e.g. individual Vγ primers. 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 date for the next Molecular and Cellular Tumor Marker PT mail-out in 2009 is:

Mail-out date	**Due Date**
October 26	November 24

Sincerely,

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