



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 2-08 Summary of results¹

April 29, 2008

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from February 25, 2008, MCTM 2-08.

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

Shipping problem: as most of you are aware we had a shipping problem, causing those labs in the western half of the country to receive their samples a week late. We again apologize for this problem. An investigation revealed that because of an incomplete software update by our vendor the address file we uploaded to our shipper erroneously and unbeknownst to us indicated that the packages were to be shipped ground instead of by air. As soon as we became aware of the problem, we sent out an email to all labs alerting them to this delay. This, however, brought to light another issue. Several of the emails bounced back because the email address we had collected during the previous PT was no longer valid. Whether that was because the recipient no longer worked there, or for some other reason we cannot tell. Furthermore, for some other labs we had a valid email address, but the recipient never forwarded it to the people in the lab actually doing the testing, indicating a problem with internal communication. While we certainly hope that this particular event does not repeat itself, there may be other reasons why we may have to get in touch with the people testing the samples. ***For this reason we always ask for an email contact address on the result form. We urge you to make sure that this address is correct and goes to the person(s) responsible for the actual testing. We can also handle multiple addresses per lab.*** Should you wish to update this information at any time between PT events, please send an email with the updated information to Susanne McHale at smchale@wadsworth.org. Thank you for your attention to this matter.

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)

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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 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, 19 out of 22 (86%) laboratories that used PCR reported a rearrangement, as did all four laboratories that used SB. Similarly, all ten labs that tested for IGK (PCR=6, SB=3) reported a rearrangement. The one lab that also tested for IGL by SB 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.

T-cell tests: All 24 laboratories that tested for TRG by PCR found no rearrangement. Four out of six (67%) labs that tested for TRB by PCR indicated a rearrangement; the other two labs reported G. Interestingly, all five labs that indicated the source of their primers used the Biomed-2 kit, and all five found a rearranged band with tube C (Table 7). However, one of those labs stated that they considered this band to be derived from an incomplete (D-J) rearrangement, and to be insufficiently correlated with neoplastic processes in the T-cell lineage. Thus, they classified the overall sample as “G” for TRB. It

would be interesting to see the opinion of others on this subject. We encourage you to email us your thoughts and we will collect and include them in our next discussion. In contrast, only two out of six labs (33%) that used SB reported a rearrangement. One laboratory also tested for TRD by SB, and found no rearrangement. Thus, there was no consensus whether the cells in this sample contained a T-cell receptor beta gene rearrangement or not.

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

IGHV mutation: Five labs tested for IGHV hypermutation, four by PCR and one by RT-PCR; four out of the five labs (80%) reported no hypermutation, and one lab reported 6.6% mutation in the IGHV3-9 family.

The results from all other tests performed were negative.

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

NYS#L/L 2:

B-cell tests: For IGH, all 27 laboratories reported no rearrangement (20= PCR, 5= SB, 2= FISH). Similarly, all six labs that tested for IgKappa (IGK) by PCR found no rearrangement, as did the three laboratories that tested for IGK by SB, and the one lab that also tested for IgLambda (IGL) by SB. 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 did not contain immunoglobulin gene rearrangements.

T-cell tests: All 25 laboratories that tested for TcRGamma (TRG) by PCR found a rearrangement, possibly in the V γ 1-8 and 10 regions that were detected by either Biomed-2 tube A or IVS mix 1, or home brew V γ 1-8 and 10 primers (Table 6). Likewise, all twelve labs that tested for TcRBeta (TRB) reported a rearrangement (6=SB, 6=PCR), possibly in the J β 1 region detected by the Biomed-2 tube A primers (Table 7). The one laboratory that tested for TcRDelta (TRD) by SB found no rearrangement. These results suggest that this sample contained cells with T-cell receptor beta and gamma gene rearrangements.

P53: One lab out of two (50%) detected a missense mutation (R273[R,C]) 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. This conclusion is in agreement with the result from Flow Cytometry, which indicated an immature thymocyte.

NYS#L/L 3:

B-cell tests: For IGH, 20 out of 21 laboratories (95%) that used PCR reported no rearrangement, as did the five labs that used SB and the two labs that used FISH. Similarly, 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 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 did not contain immunoglobulin gene rearrangements.

T-cell tests: All 25 laboratories that tested for TcRGamma (TRG) by PCR found a rearrangement, possibly in the V γ 1-8 regions detected by the Biomed-2 tube A, the IVS mix 1 or home brew V γ 1-8 primers (Table 6). Likewise, all eleven labs that tested for TcRBeta (TRB) reported a rearrangement (5=SB, 6=PCR), possibly in the J β 1 region detected by the Biomed-2 tube A primers (Table 7). The one laboratory that tested for TcRDelta (TRD) by SB found no rearrangement. These results suggest that this sample contained cells with T-cell receptor beta and gamma gene rearrangements.

P53: One lab out of two (50%) detected three missense mutations (R175[H,R]; R248[Q,R]; R282[R,W]) 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. This conclusion is in agreement with the result from Flow Cytometry, which indicated a mature thymocyte (helper-inducer) with an aberrant lack of expression of CD2 and sCD3.

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 contained the EBV, and L/L 2 and 3 possibly contained a P53 mutation. 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 10, 12, and 12 μ g in 5 ml specimen to a maximum of 21250, 6050, and 8050 μ g, respectively, corresponding to an about 2100- to 500-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 3, 6.3, and 2.5 μ g to 975, 505, and 571 μ g, respectively, corresponding to an about 325- to 80-fold difference between lowest and highest yield for each sample. Two labs reported yields of 975 and 900 μ g RNA for L/L 1, which is 720 μ g more than the next highest RNA yield that was 180 μ g. 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. 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 and may be against some lab's specimen acceptance policy. However, we have not detected substantial problems with results because of that

(except this time because of the shipping problems discussed earlier). 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. For example, there were two labs that entered their JAK2 and IGHV results in the RT-PCR column; since they used genomic DNA as their starting material (according to their SOP), the results should have been written in PCR column. Thus, 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. 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

June 23

October 27

Due Date

July 22

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