

STATE OF NEW YORK DEPARTMENT OF HEALTH

Wadsworth Center

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Molecular Oncology Proficiency Test Program MODE 10-04 Summary of results¹

December 7, 2004

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular Oncology from October 2004, MODE 10-04.

Samples: all laboratories received three (3) different specimens prepared by Wadsworth Center personnel. Cultured cells were added in various ratios to normal donor blood the day of the mail-out.

Evaluation: Laboratories were asked to perform those molecular assays that they routinely perform, based on the results from a preliminary analysis by Flow Cytometry (if applicable). The attached tables summarize the results and methods that were used by participating laboratories. A consensus result of **G**(ermline) or **R**(erranged) is also indicated where possible. A total of 30 laboratories participated. However, one laboratory did not perform any tests because of insufficient DNA obtained by their DNA isolation method. As this was a purely educational event, no grades were assigned, nor do you receive an individualized result form. However, each of you should compare your results to the group consensus, and if there is a discrepancy, investigate the possible causes. This is especially recommended for those labs whose results from a given test or assay were opposite to the majority of the results from all the other labs.

NYS#L/L 1: For IgH, sixteen laboratories reported results from PCR analysis, whereas four labs performed Southern blot (SB). Of those, all 16 (100%) using PCR reported finding no rearrangement for IgH, whereas by Southern blot, two of four (50%) detected a rearrangement, one found no rearrangement and one found an indeterminate result. Interestingly, those two that found a rearrangement by SB failed to detect this by PCR. Only three labs also tested for IgKappa and/or IgLambda, finding no rearrangement.

Nineteen laboratories tested for and all (100%) reported finding a rearrangement of TcRGamma by PCR, and three out of five (60%) that tested for TcRBeta found a rearrangement by PCR. Four of the six (67%) that used SB reported a rearrangement of TcRBeta. One laboratory also tested, but found no rearrangement of TcRDelta. All ten laboratories (100%) that

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.

tested for BCL2-IGHJ Mbr reported no rearrangement of this locus, whereas one of six (16%) reported a rearrangement for BCL2-IGHJ mcr. Results from all other tests performed were negative, i.e., did not detect a rearrangement, or found no evidence for viral sequences.

Overall, these results give a general consensus of a T-cell clone, consistent with the known genotype of the **Jurkat** T-cell line used to spike the blood samples. The Jurkat cell line was established from the peripheral blood of a 14-year old boy with acute T-cell leukemia (from ATCC catalog, #TIB-152).

NYS#L/L 2: For IgH, exactly half, i.e., ten of the 20 laboratories that used PCR, reported a rearrangement. Of those, 3 used the Biomed-2 primers, 4 used a home brew assay, but did not indicate what primers they used, 2 used a home brew, either with Framework 1 and 3, or Framework 2 primers, respectively, and 1 used the InVivoScribe (IVS) FR3 primers. Of the ten labs that did not find an IgH rearrangement by PCR, 7 labs used a home brew, but did not indicate what primers they used, and 3 used a home brew with Framework 3 primers. Thus, it appears that the choice of the primers played an important role in whether a lab detected an IgH rearrangement for this sample. Five of six laboratories (83%) reported rearrangement of IgH by SB, whereas the one that found no rearrangement by SB and also did not detect a rearrangement by PCR, using FR3 primers. One laboratory reported rearrangement of IgL by PCR, whereas one other laboratory reported no rearrangement of IgL by SB.

Nine laboratories tested for and all (100%) reported finding no rearrangement of TcRBeta, either by SB or PCR. Similarly, out of seventeen labs that tested for TcRGamma by PCR, only one found a rearrangement using a home brew method. Two laboratories also tested for TcRDelta by SB and found it to be in its germline configuration. Ten of eleven laboratories (91%) that tested for BCL2-IGHJ Mbr reported a translocation, and one out of the six labs (16%) that also tested for BCL2-IGHJ mcr reported a rearrangement using a home brew method. The t(14:18) translocation was also found by FISH by four labs, whereas one lab found no translocation by this method. No other gene rearrangements were detected, and no evidence for the presence of viral sequences was found.

In aggregate, these results indicate that the sample contained a B-cell clone with a t(14:18) translocation, which is consistent with the reported genotype for the **RL** cell line used to spike the blood. RL is a human non-Hodgkin's lymphoma B cell line originated in 1983. RL cells have been reported to be Epstein-Barr virus genome negative (from ATCC catalog, #CRL-2261).

NYS#L/L 3: Seventeen of nineteen laboratories (89%) that tested for IgH by PCR reported finding a rearrangement, and four of six laboratories (67%) reported finding a rearrangement of IgH by SB. Three of four (75%) laboratories also reported rearrangement of IgK by PCR, and two of four by SB. The one laboratory that also tested for IgL by SB found no rearrangement. Among the 17 labs that found a rearrangement of IgH by PCR, 3 used the Biomed-2 primers, 2 the IVS primers (1 x FR3, 1 x FR not given), and 11 used a home brew, but did not indicate the primers used. The two labs that did not find a rearrangement both used a home brew method, one of which used primers targeted to FR3.

All sixteen laboratories (100%) that tested for TcRG by PCR found no rearrangement as did the five that used PCR to test for TcRB. Furthermore, four of five (80%) labs reported no

rearrangement of TcRB by SB, whereas one lab obtained an indeterminate result. Two laboratories tested for, but found no rearrangement of TcRD. All other tests performed to detect gene rearrangements were negative. Four labs tested for and found the sample to be EBV positive.

In aggregate, these results indicate the presence of a B-cell clone with EBV, consistent with the reported genotype for the **Raji** cell line used to spike the blood. The Raji line of lymphoblast-like cells was established in 1963 from a Burkitt's lymphoma of the left maxilla of an 11-year-old Black male and is EBNA positive (from ATCC catalog, #CCL-86).

One laboratory did not indicate the methods that were used for each assay. Please note that all method-related information should be written in the right-most column of the result sheet; this includes the reagents and/or kits used. Furthermore, please note that one the submission deadline has passed, no changes can me made.

Several labs commented on the short time frame given to perform the tests. In recognition of these concerns, the time allotted for future events will be extended to one month. The tentative schedule for 2005 is as follows (note that the final schedule will also be posted at <http://www.wadsworth.org/labcert/clep/PT/PTschedule.htm>)

| Mail-out date | Post-mark date |
|----------------------|-----------------------|
| Feb 22 | March 25 |
| Jun 21 | July 22 |
| Oct 11 | Nov 11 |

If you have any questions, comments or suggestions, you may contact me by phone or email at 518-474-2088 or schneid@wadsworth.org.

Sincerely

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PT_MODE_summary_10-04
NYS#LL 1

| Sample | NYS#L/L 1 | | | | PCR | | | | RT-PCR | | | | FISH | | | | Method used | SB | PCR | RT-PCR (qualitative) | RT-PCR (quantitative) | FISH |
|-----------------------|-----------|---|-----|----------|-----|----|-----|----------|--------|---|-----|------|------|---|-----|----------|-------------------------|---|--|----------------------|-----------------------|------|
| | + | - | ind | Cons | + | - | ind | Cons | + | - | ind | Cons | + | - | ind | Cons | | | | | | |
| Ig H | 2 | 1 | 1 | ? | | 16 | | G | | | | | | | | | Dako (2), home brew (1) | IVS (1), Biomed (2), home brew (12), Invitrogen (1) | | | | |
| Ig kappa | | 2 | | G | | 1 | | G | | | | | | | | | home brew (1) | Biomed (1) | | | | |
| Ig lambda | | 1 | | G | | | | | | | | | | | | | home brew (1) | | | | | |
| TcR beta | 4 | 2 | | R | 3 | 2 | | ? | | | | | | | | | Dako (3), home brew (1) | Biomed (1), home brew (4) | | | | |
| TcR gamma | | | | | 19 | | | R | | | | | | | | | | IVS (1), Biomed (2), home brew (15), Invitrogen (1) | | | | |
| TcR delta | | 1 | | G | | | | | | | | | | | | | not indicated | | | | | |
| Bcl2 t(14;18) MBR | | | | | | 9 | | G | | | | | 1 | | | G | | Biomed (2), home brew (7) | | | not indicated | |
| MBR 3' | | | | | | 1 | | G | | | | | | | | | | | | | | |
| mcr | | | | | 1 | 5 | | G | | | | | | | | | | Biomed (1), home brew (5) | | | | |
| Bcl1 t(11;14) | | | | | | 5 | | G | | | | | 1 | | | G | | home brew (5) | | | not indicated | |
| Bcr/abl t(9;22) Major | | 1 | | G | | 2 | | G | 11 | 1 | G | | 2 | | | G | not indicated | Hemavision-15;17 (1) | Roche (2), home brew (8), Invitrogen (1) | | Vysis (1) | |
| Minor | | | | | | 2 | | G | 10 | 1 | G | | | | | | | Hemavision-15;17 (1) | Roche (2), home brew (8) | | | |
| PML/RARa Long | | | | | | 2 | | G | 2 | | G | | 1 | | | G | | Hemavision-15;17 (1), home brew (1) | home brew (2) | | not indicated | |
| Short | | | | | | 2 | | G | 2 | | G | | | | | | | Hemavision-15;17 (1), home brew (1) | home brew (2) | | | |
| Variable | | | | | | 1 | | G | 2 | | G | | | | | | | Hemavision-15;17 (1), home brew (1) | home brew (1) | | | |
| c-myc t(8;14) | | 1 | | G | | | | | | | | | 1 | | | G | home brew (1) | | | | not indicated | |
| AML1/ETO t(8;21) | | | | | | | | | 4 | | G | | 1 | | | G | | | Roche (1), home brew (3) | | not indicated | |
| NPM/ALK t(2;5) | | | | | | | | | 3 | | G | | 2 | | | G | | | home brew (3) | | Vysis (1) | |
| TEL/AML1 t(12;21) | | | | | | | | | 2 | | G | | | | | | | | home brew (2) | | | |
| EBV | | | | | | 4 | | - | | | | | | | | | | | home brew (2), Roche/ASR (1) | | | |
| KSHV/HHV8 | | | | | | 2 | | - | | | | | | | | | | | home brew (2) | | | |
| HTLV1 | | | | | | 1 | | - | | | | | | | | | | | home brew (1) | | | |
| CBFB INV(16) | | | | | | | | | | | | | | | | | | | | | | |

Note: G or R for consensus was based on the majority of responses. However, if the reported results were evenly split or close to evenly split with a majority of 1, this was marked with a "?"

**PT_MODE_summary_10-04
NYS#LL 2**

| Sample | NYS#L/L 2 | | | | | | | | | | | | | | | | Method used | RT-PCR (qualitative) | RT-PCR (quantitative) | FISH | |
|-----------------------|-----------|---|-----|------|-----|----|-----|------|--------|---|-----|------|------|---|-----|------|-------------------------|---|--------------------------|-----------------------|---------------|
| | SB | | | | PCR | | | | RT-PCR | | | | FISH | | | | | | | | |
| Assay | + | - | ind | Cons | + | - | ind | Cons | + | - | ind | Cons | + | - | ind | Cons | SB | PCR | RT-PCR (qualitative) | RT-PCR (quantitative) | FISH |
| Ig H | 5 | 1 | | R | 10 | 10 | ? | | | | | | | | | | Dako (2), home brew (2) | IVS (1), Biomed (3), home brew (15), Invitrogen (1) | | | |
| Ig kappa | 2 | 1 | | ? | 2 | 1 | ? | | | | | | | | | | home brew (2) | Biomed (2), home brew (2) | | | |
| Ig lambda | | 1 | | G | 1 | | R | | | | | | | | | | home brew (1) | home brew (1) | | | |
| TcR beta | | 5 | | G | | 4 | G | | | | | | | | | | Dako (3), home brew (1) | Biomed (1), home brew (3) | | | |
| TcR gamma | | | | | 1 | 16 | G | | | | | | | | | | | Biomed (2), home brew (14), Invitrogen (1) | | | |
| TcR delta | | 2 | | G | | | | | | | | | | | | | not indicated | home brew (1) | | | |
| Bcl2 t(14;18) MBR | | | | | 10 | 1 | R | | | | | | 4 | 1 | | | | Biomed (2), home brew (9) | | | Vysis (2) |
| MBR 3' | | | | | | 1 | G | | | | | | | | | | | | | | |
| mcr | | | | | 1 | 5 | G | | | | | | | | | | | Biomed (1), home brew (5) | | | |
| Bcl1 t(11;14) | | | | | | 5 | G | | | | | | | 1 | | | | home brew (5) | | | not indicated |
| Bcr/abl t(9;22) Major | | 1 | | G | | 2 | G | | 10 | 1 | G | | 2 | | G | | not indicated | Hemavision-15;17 (1) | Roche (2), home brew (8) | | Vysis (2) |
| Minor | | | | | | 2 | G | | 10 | 1 | G | | | | | | | Hemavision-15;17 (1) | Roche (2), home brew (8) | | |
| PML/RARa Long | | | | | | 2 | G | | 2 | | G | | 1 | | G | | | Hemavision-15;17 (1), home brew (1) | home brew (2) | | not indicated |
| Short | | | | | | 2 | G | | 2 | | G | | | | | | | Hemavision-15;17 (1), home brew (1) | home brew (2) | | |
| Variable | | | | | | 1 | G | | 2 | | G | | | | | | | Hemavision-15;17 (1) | home brew (2) | | |
| c-myc t(8;14) | | 1 | | G | | | | | | | | | | 1 | G | | home brew (1) | | | | LSI |
| AML1/ETO t(8;21) | | | | | | | | | 4 | | G | | 1 | | G | | | | Roche (1), home brew (3) | | not indicated |
| NPM/ALK t(2;5) | | | | | | | | | 3 | | G | | 2 | | G | | | | home brew (3) | | not indicated |
| TEL/AML1 t(12;21) | | | | | | | | | 2 | | G | | | | | | | | home brew (2) | | |
| EBV | | | | | 4 | | - | | | | | | | | | | | home brew (2), Roche/ASR (1) | | | |
| KSHV/HHV8 | | | | | 2 | | - | | | | | | | | | | | home brew (2) | | | |
| HTLV1 | | | | | 1 | | - | | | | | | | | | | | home brew (1) | | | |
| CBFB INV(16) | | | | | | | | | | | | | | | | | | | | | |

Note: G or R for consensus was based on the majority of responses. However, if the reported results were evenly split or close to evenly split with a majority of 1, this was marked with a "?"

**PT_MODE_summary_10-04
NYS#LL 3**

| Sample | NYS#L/L 3 | | | | PCR | | | | RT-PCR | | | | FISH | | | | Method used | | | | |
|-----------------------|-----------|---|-----|------|-----|----|-----|----------|--------|---|----------|------|------|---|-----|----------|-------------------------|---|--------------------------|-----------------------|---------------|
| | + | - | ind | Cons | + | - | ind | Cons | + | - | ind | Cons | + | - | ind | Cons | SB | PCR | RT-PCR (qualitative) | RT-PCR (quantitative) | FISH |
| Ig H | 4 | 1 | 1 | R | 17 | 2 | | R | | | | | | | | | Dako (3), home brew (2) | IVS (2), Biomed (3), home brew (14), Invitrogen (1) | | | |
| Ig kappa | 2 | 1 | 1 | ? | 3 | | 1 | R | | | | | | | | | home brew (1) | Biomed (2), home brew (1), IVS (1) | | | |
| Ig lambda | | 1 | | G | | | | | | | | | | | | | home brew (1) | | | | |
| TcR beta | | 4 | 1 | G | | 5 | | G | | | | | | | | | Dako (2), home brew (2) | Biomed (1), home brew (4) | | | |
| TcR gamma | | | | | | 16 | | G | | | | | | | | | | Biomed (2), home brew (13), Invitrogen (1) | | | |
| TcR delta | | 2 | | G | | | | | | | | | | | | | home brew (1) | | | | |
| Bcl2 t(14;18) MBR | | | | | | 10 | | G | | | | | | 2 | | G | | Biomed (2), home brew (8) | | | Vysis (1) |
| MBR 3' | | | | | | 1 | | G | | | | | | | | | | | | | |
| mcr | | | | | | 6 | | G | | | | | | | | | | Biomed (1), home brew (5) | | | |
| Bcl1 t(11;14) | | | | | | 5 | | G | | | | | | 1 | | G | | home brew (5) | | | not indicated |
| Bcr/abl t(9;22) Major | | 1 | | G | | 2 | | G | 10 | 1 | G | | | 2 | | G | not indicated | Hemavision-15;17 (1) | Roche (2), home brew (8) | | Vysis (1) |
| Minor | | | | | | 2 | | G | 10 | 1 | G | | | | | | | Hemavision-15;17 (1) | Roche (2), home brew (8) | | |
| PML/RARa Long | | | | | | 2 | | G | 2 | | G | | | 1 | | G | | Hemavision-15;17 (1), home brew (1) | home brew (2) | | |
| Short | | | | | | 2 | | G | 2 | | G | | | | | | | Hemavision-15;17 (1), home brew (1) | home brew (2) | | |
| Variable | | | | | | 1 | | G | 2 | | G | | | | | | | Hemavision-15;17 (1) | home brew (2) | | |
| c-myc t(8;14) | | 1 | | G | | | | | | | | | | 2 | | G | home brew (1) | | | | LSI |
| AML1/ETO t(8;21) | | | | | | | | | 1 | 3 | G | | | 1 | | G | | | Roche (1), home brew (3) | | not indicated |
| NPM/ALK t(2;5) | | | | | | | | | | 3 | G | | | 1 | | G | | | home brew (3) | | not indicated |
| TEL/AML1 t(12;21) | | | | | | | | | | 2 | G | | | | | | | | home brew (2) | | |
| EBV | | | | | 4 | | | + | | | | | | | | | | home brew (2), Roche/ASR (1) | | | |
| KSHV/HHV8 | | | | | | 2 | | - | | | | | | | | | | home brew (2) | | | |
| HTLV1 | | | | | | 1 | | - | | | | | | | | | | home brew (1) | | | |
| CBFB INV(16) | | | | | | | | | | | | | | | | | | | | | |

Note: G or R for consensus was based on the majority of responses. However, if the reported results were evenly split or close to evenly split with a majority of 1, this was marked with a "?"