NEW YORK
state department of
HEALTH

Nirav R. Shah, M.D., M.P.H.
Commissioner

Sue Kelly
Executive Deputy Commissioner

# Molecular and Cellular Tumor Marker Proficiency Test Event
# MCTM 10-2013
# Summary of results[1]

December 17, 2013

Dear Laboratory Director,

Below is a summary and discussion of the New York State Molecular and Cellular Tumor Markers proficiency test event MCTM 10-2013 from October 22, 2013.

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. In Table 1, a consensus interpretation is shown of **R**: rearranged/clonal band detected; **G**: germline/no clonal band detected; **WT**: wild-type; **MUT**: mutated; **NEG**: negative or not detected; **POS**: positive or detected; **O**: oligoclonal; **N**: no clonal band or fusion product detected. For IGHV only: **H**: clonal band detected and hypermutated; **U**: clonal band detected, but not hypermutated; **I** (Indeterminate) is shown if no consensus was reached because less than three labs performed a test, or if the concordance between labs was less than 80%. Please note that in a change from previous summary tables, only the all method consensus is shown.

Each lab will receive a personalized result sheet by regular mail that shows your lab's results in comparison to the all lab consensus (if any) 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 for each sample. 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, i.e. produced a consensus, and the numerator is the number of samples for which you agreed 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, but agreed with the consensus for only one of them. The assay 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

[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

that were evaluable. Assays for which no clear consensus was obtained or for which you were unable to obtain a clear result, 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 x 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 in case of apparently false positives.

**NYS#L/L 2013-04 (Table 1)**

B-cell tests: All 27 laboratories that tested for IgH reported no rearrangement, as did the fifteen laboratories that also tested for IgK. Of the twelve laboratories that tested for IGH/BCL2 only one (8%) reported a translocation specifically with the Biomed-2 tube C primers that target the minor breakpoint. However, the four other labs that also targeted this region did not detect this fusion. No fusions between IGH and CCND1 were detected. For IGHV eleven out of twelve labs (92%) reported no clonal band, whereas one lab reported a clonal band that was not hypermutated. Thus, there was a consensus that this sample did not contain cell clones with immunoglobulin gene rearrangements or translocations involving the IGH locus.

T-cell tests: 22 out of 25 laboratories (88%) that tested for TRG found no rearrangement as did nine out of fourteen labs (64%) that tested for TRB; however, two labs reported a rearrangement for TRB and one lab for TRG and three and two labs, respectively, reported an indeterminate result for either TRB and/or TRG. These results suggest that this sample did not contain a major clone with T-cell receptor gene rearrangements, though a minor clone cannot be excluded.

Other translocations: Of all the translocations/fusions that laboratories tested for, only AML1/ETO was consistently detected by all eight labs testing for it, suggesting that this sample contains a clone with the t(8;21) translocation.

Various mutations (Table 7): Six out of eight (75%) labs detected a KIT mutation, c. 2466T>A; p. N822K in exon 17. The other two labs only test specifically for D816V and thus would not have detected the N822K mutation in this sample. Four out of five labs (81%) detected a TP53 mutation, c.743G>A; p.R248Q, whereas one lab reported an indeterminate mutation. Lastly, one out of seven labs (14%) detected a FLT3 TKD mutation. No other mutations were detected in any gene.

EBV: One out of five labs (20%) reported the presence of EBV sequences.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained an AML clone with a t(8;21) translocation, which is consistent with the results from Flow Cytometry, which indicated the presence of an immature myeloid progenitor cell that expressed surface CD4, CD13, CD15, CD33, CD34 and CD45.

**NYS#L/L 2013-05 (Table 1)**

B-cell tests: For IGH and IGK, there was unanimous agreement that these genes were rearranged. Rearrangements in IGH were detected with all primers irrespective of their provenance, except for Biomed-2 tube E that targets the DH7 region. Rearrangements in IGK too were detected with all primers (Table 3). No lab reported a fusion between IGH and BCL2 or CCND1, respectively. Twelve labs tested for IGHV hypermutation and all assigned it to the IGHV4-34 family; the mutation rate ranged from 1.9 to 2.6%, which resulted in ten hypermutation calls (83%), one unmutated and one indeterminate call. In conclusion, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements and IGHV hypermutation.

T-cell tests: All laboratories that tested for TRB or TRG found no rearrangement except for one indeterminate result for TRG; these results suggest that this sample did not contain cell clones with TRB and/or TRG gene rearrangements.

Other translocations: No translocations/fusions were detected.

Various mutations (Table 7): 5/5 labs detected the TP53 mutation, c.AT760-761GA; p.I254N, and 1/30 detected the JAK2 V617F mutation. No other mutations were detected in any gene.

EBV: One out of five labs (20%) reported the presence of EBV sequences.

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 a TP53 mutation. The overall result is consistent with the results from Flow Cytometry, which indicated a mature B-cell phenotype (CD10, CD19, $CD20^{dim}$, CD45, and HLA-DR) with lambda clonality.

**NYS#L/L 2013-06 (Table 1)**

B-cell tests: 25 out of 27 laboratories (93%) that tested IGH reported no rearrangement, one lab reported a rearrangement in framework 2, and one reported an indeterminate result. In contrast, eleven out of fourteen laboratories (78%) that tested IGK reported a rearrangement with one lab reporting the result as oligoclonal. This result is just short of the 80% needed for a consensus. Further studies are needed to determine whether this sample indeed contained a true clonal population of cells with IGK rearrangement, especially since, with two exceptions, only the Biomed-2 tube B primers detected a rearrangement. No lab reported a translocation involving the IGH/BCL2 or IGH/CCND1 loci. Thus, there was a consensus that this sample did not contain cell clones with immunoglobulin gene rearrangements or translocations involving the IGH locus, whereas the presence of an IGK rearrangement needs further confirmation.

T-cell tests: All 25 laboratories that tested for TRG reported a rearrangement and twelve out of fourteen labs (86%) that tested for TRB found a rearrangement. Thus, there was general consensus that this sample exhibited TRG and TRB rearrangements.

Other translocations: No translocations/fusions were detected.

Various mutations (Table 7): Five labs reported two or three concurrent TP53 mutations, c.743G>A, p.R248Q; c.800G>T, p.R267L; c.818G>A, p.R273H; and a polymorphism, c.215C>G; p.P72R. Two labs also reported a MPL mutation, c.1489G>A; p.A497T, and three labs reported the PIK3CA, p.E545D mutation. One lab reported a BRAF mutation in codons 599-602 in exon 15 but did not indicate the exact sequence variation. No other mutations were detected.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a T-cell clone with TRG and TRB rearrangements. Whether the sample contained a second, possibly minor clone with an IGK rearrangement or whether both rearrangements are present in the same cells cannot be determined without further investigation. Furthermore, it appears that this sample contained multiple, possibly oncogenic mutations. The overall interpretation of T-cell clonality is consistent with the results from Flow Cytometry, which indicated an immature $CD3^-$ double-positive T cell (CD2, CD4, CD5, CD7, and CD8).

## General comments

The attached tables show summaries of the results both overall (Table 1) as well as for each individual primer mix for the B- and T-cell tests (Tables 2-6). Furthermore, Table 7 shows a summary of the mutation results, and Tables 8 shows summaries of the methods and reagents used for most of the tests. Figure 1 shows the DNA and RNA yield distributions for the three samples. Again the differences in yield are large ranging from 670- to over 13750-fold, raising the question whether everybody reported their results the same way. Please make sure that you report the DNA and RNA yields in microgram (µg) and based on the actual volume of the original blood sample from which you isolated the DNA and RNA. Do not report the volume as the volume in which you eluded the nucleic acid into. 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, we would like to add some general comments. You really need to follow our instructions for filling out the result form, or we cannot guarantee correct evaluation of your results. **You must select the overall result in the first column**, as it is this result that is used in the evaluation. Then fill in or select the part of the additional information as appropriate. Also please make sure that you choose the correct method where there is a choice. If your starting material is DNA you must choose PCR. If your starting material is RNA, you must choose RT-PCR. 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. Furthermore, we ask that if you obtain your primers/kits from

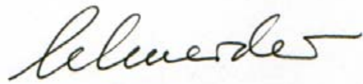
InVivoScribe you correctly identify the source as IVS (not Biomed-2) (identified as gene rearrangement assays in their catalog) or IVS (Biomed-2) (identified as gene clonality assays in their catalog); for the purpose of this PT evaluation they are not considered lab developed even if you obtain the individual primer tubes separately as ASR reagents instead of as part of a RUO kit. This will make it easier to compare the performance of individual primer mixes. 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 Dr. Rong Yao at (518) 474-1744 or yaor@wadsworth.org or Ms. Susanne McHale at (518) 486-5775 or smchale@wadsworth.org.

Please note there was a change to two Molecular and Cellular Tumor Marker PT mail-outs in 2014, with the next one being:

| **Mail-out date** | **Due Date** |
| --- | --- |
| March 18, 2014 | April 16, 2014 |
| October 28, 2014 | November 26, 2014 |

Sincerely,



Erasmus Schneider, Ph.D.
Director, Oncology Section
Clinical Laboratory Evaluation Program
Wadsworth Center, Room E604
Empire State Plaza
Albany, NY 12201-0509

**New York State Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 10-2013**
**Table 1: Summary of results**

| Assay / Sample | L/L 2013-04 | | | | | L/L 2013-05 | | | | | L/L 2013-06 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R/H | G/U | I | O/N | Cons[#] | R/H | G/U | I | O/N | Cons[#] | R/H | G/U | I | O/N | Cons[#] |
| IGH | | 26 | 1 | | G | 27 | | | | R | 1 | 25 | 1 | | G |
| IGK | | 15 | | | G | 15 | | | | R | 10 | 4 | | 1 | I |
| TRB | 2 | 9 | 3 | | G | | 14 | | | G | 12 | 2 | | | R |
| TRG | 1 | 22 | 2 | | G | | 24 | 1 | | G | 25 | | | | R |
| IGHV | | 1 | | 11 | N | 10 | 1 | 1 | | H | | 1 | | 11 | N |
| | POS | NEG | I | | | POS | NEG | I | | | POS | NEG | I | | |
| IGH/BCL2 | 1 | 11 | | | NEG | | 11 | 1 | | NEG | | 12 | | | NEG |
| IGH/CCND1 | | 7 | | | NEG | | 7 | | | NEG | | 7 | | | NEG |
| IGH/MYC | | | | | | | | | | | | | | | |
| | MUT* | WT* | I | | | MUT* | WT* | I | | | MUT* | WT* | I | | |
| JAK2 V617F | | 30 | | | WT | 1 | 29 | | | WT | | 30 | | | WT |
| JAK2 Exon 12 | | 9 | | | WT | | 9 | | | WT | | 9 | | | WT |
| MPL | | 11 | | | WT | | 11 | | | WT | 2 | 9 | | | WT |
| FLT3 ITD | | 8 | | | WT | | 8 | | | WT | | 8 | | | WT |
| FLT3 TKD | 1 | 6 | | | WT | | 7 | | | WT | | 7 | | | WT |
| NPM1 | | 15 | | | WT | | 15 | | | WT | | 15 | | | WT |
| CEBPA | | 7 | | | WT | | 7 | | | WT | | 7 | | | WT |
| IDH1 | | 6 | | | WT | | 6 | | | WT | | 6 | | | WT |
| IDH2 | | 4 | | | WT | | 4 | | | WT | | 4 | | | WT |
| KIT | 6 | 2 | | | I | | 7 | | | WT | | 7 | | | WT |
| | POS | NEG | I | | | POS | NEG | I | | | POS | NEG | I | | |
| BCR/ABL1 p210 | | 28 | | | NEG | | 28 | | | NEG | | 28 | | | NEG |
| BCR/ABL1 p190 | | 26 | | | NEG | | 26 | | | NEG | | 26 | | | NEG |
| BCR/ABL1 p210/p190 | | 5 | | | NEG | | 5 | | | NEG | | 5 | | | NEG |
| | MUT* | WT* | I | N | | MUT* | WT* | I | N | | MUT* | WT* | I | N | |
| ABL Kinase domain | | 2 | | 5 | I | | 2 | | 5 | I | | 2 | | 5 | I |
| | POS | NEG | I | | | POS | NEG | I | | | POS | NEG | I | | |
| PML/RARA Long | | 8 | | | NEG | | 8 | | | NEG | | 8 | | | NEG |
| PML/RARA short | | 8 | | | NEG | | 8 | | | NEG | | 8 | | | NEG |
| PML/RARA variable | | 2 | | | I | | 2 | | | I | | 2 | | | I |
| PML/RARA L/S/V | | | | | | | | | | | | | | | |
| AML1/ETO | 8 | | | | POS | | 8 | | | NEG | | 8 | | | NEG |
| NPM1/ALK | | | | | | | | | | | | | | | |
| ETV6/RUNX1 | | 3 | | | NEG | | 3 | | | NEG | | 3 | | | NEG |
| CBFB/MYH11 | | 4 | | | NEG | | 4 | | | NEG | | 4 | | | NEG |
| TCF3/PBX1 | | 1 | | | I | | 1 | | | I | | 1 | | | I |
| MLL/AF4 | | 2 | | | I | | 2 | | | I | | 2 | | | I |
| | MUT* | WT* | I | | | MUT | WT | I | | | MUT* | WT* | I | | |
| TP53 | 4 | | 1 | | MUT | 5 | | | | MUT | 5 | | | | MUT |
| KRAS | | 10 | | | WT | | 10 | | | WT | | 10 | | | WT |
| NRAS | | 5 | | | WT | | 5 | | | WT | | 5 | | | WT |
| HRAS | | 3 | | | WT | | 3 | | | WT | | 3 | | | WT |
| BRAF | | 11 | | | WT | | 11 | | | WT | 1 | 10 | | | WT |
| | POS | NEG | I | | | POS | NEG | I | | | POS | NEG | I | | |
| EBV | 1 | 4 | | | NEG | 1 | 4 | | | NEG | | 5 | | | NEG |
| Interpretation: | Acute Myeloid Leukemia with t(8;21) translocations resulting in the AML1/ETO fusion gene, and KIT mutation | | | | | B-Cell Lymphoproliferative Disdorder with mature B-cell phenotype | | | | | T-lymphoblastic leukemia with clonal TCRG rearrangment; possibility of a minor clone with IGK rearrangement cannot be excluded; also multiple mulitple single nucleotid variants in multiple genes detected. | | | | |
| Comments | | | | | | | | | | | | | | | |

R: rearranged/clonal band detected; G: germline/no clonal band detected; O: oligoclonal; For IGHV only: H: clonal band detected and hypermutated; U: clonal band detected, but not hypermutated; N: no clonal band detected.
MUT: mutated; WT: wild-type; N: no fusion product detected; NEG: neagtive or not detected; POS: positive or detected; I: indeterminate, a clear interpretation is not possible.
[#]Consensus based on ≥80% concordance; I if no consensus or <3 results
*For details of which exons/codons were analyzed see table 7.

**Table 2. Summary for IGH primer mixes**

| | L/L 2013-04 | | | L/L 2013-05 | | | L/L 2013-06 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **R** | **G** | **cons** | **R** | **G** | **cons** | **R** | **G** | **cons** |
| LDT FR 1 | 0 | 2 | **I** | 2 | 0 | **I** | 0 | 2 | **I** |
| LDT FR 2 | 0 | 8 | **G** | 8 | 0 | **R** | 0 | 8 | **G** |
| LDT FR 3 | 0 | 9 | **G** | 9 | 0 | **R** | 0 | 9 | **G** |
| Biomed-2 Tube A | 0 | 11 | **G** | 11 | 0 | **R** | 0 | 11 | **G** |
| Biomed-2 Tube B | 0 | 12 | **G** | 12 | 0 | **R** | 1 | 11 | **G** |
| Biomed-2 Tube C | 0 | 11 | **G** | 11 | 0 | **R** | 0 | 11 | **G** |
| Biomed-2 Tube D | 0 | 3 | **G** | 2 | 0 | **I** | 0 | 3 | **G** |
| Biomed-2 Tube E | 0 | 4 | **G** | 0 | 3 | **G** | 0 | 4 | **G** |
| IVS FR 1 | 0 | 6 | **G** | 6 | 0 | **R** | 0 | 6 | **G** |
| IVS FR 2 | 0 | 8 | **G** | 8 | 0 | **R** | 0 | 8 | **G** |
| IVS FR 3 | 0 | 9 | **G** | 9 | 0 | **R** | 0 | 9 | **G** |

**Table 3. Summary for IGK primer mixes**

| | L/L 2013-04 | | | L/L 2013-05 | | | L/L 2013-06 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **R** | **G** | **cons** | **R** | **G** | **cons** | **R** | **G** | **cons** |
| LDT Tube A | 0 | 6 | **G** | 4 | 1 | **R** | 1 | 3 | **I** |
| LDT Tube B | 0 | 6 | **G** | 4 | 1 | **R** | 0 | 4 | **G** |
| Biomed-2 Tube A | 0 | 11 | **G** | 11 | 1 | **R** | 1 | 11 | **G** |
| Biomed-2 Tube B | 0 | 11 | **G** | 12 | 0 | **R** | 11 | 2 | **R** |

**Table 4. Summary for TRB primer mixes**

| | L/L 2013-04 | | | L/L 2013-05 | | | L/L 2013-06 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **R** | **G** | **cons** | **R** | **G** | **cons** | **R** | **G** | **cons** |
| LDT Tube A | 0 | 2 | **I** | 0 | 3 | **G** | 1 | 2 | **I** |
| LDT Tube B | 1 | 1 | **I** | 0 | 3 | **G** | 3 | 0 | **R** |
| Biomed-2 Tube A | 1 | 11 | **G** | 0 | 12 | **G** | 1 | 11 | **G** |
| Biomed-2 Tube B | 1 | 9 | **G** | 0 | 12 | **G** | 9 | 3 | **I** |
| Biomed-2 Tube C | 0 | 9 | **G** | 0 | 10 | **G** | 0 | 9 | **G** |

**Table 5. Summary for TRG primer mixes**

| | L/L 2013-04 | | | L/L 2013-05 | | | L/L 2013-06 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **R** | **G** | **cons** | **R** | **G** | **cons** | **R** | **G** | **cons** |
| LDT Vy1-8 | 0 | 7 | **G** | 0 | 7 | **G** | 7 | 0 | **R** |
| LDT Vy9 | 0 | 6 | **G** | 0 | 6 | **G** | 1 | 5 | **G** |
| LDT Vy10 | 0 | 4 | **G** | 0 | 4 | **G** | 1 | 3 | **I** |
| LDT Vy11 | 0 | 3 | **G** | 0 | 3 | **G** | 1 | 2 | **I** |
| Biomed-2 Tube A | 0 | 9 | **G** | 0 | 10 | **G** | 11 | 0 | **R** |
| Biomed-2 Tube B | 0 | 10 | **G** | 0 | 11 | **G** | 1 | 9 | **G** |
| IVS Mix 1 | 0 | 3 | **G** | 0 | 3 | **G** | 2 | 0 | **I** |
| IVS Mix 2 | 0 | 3 | **G** | 0 | 3 | **G** | 1 | 1 | **I** |
| IVS v2.0 | 0 | 3 | **G** | 0 | 3 | **G** | 3 | 0 | **R** |

**Table 6. Summary for BCL2 primer mixes**

| | L/L 2013-04 | | | L/L 2013-05 | | | L/L 2013-06 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **POS** | **NEG** | **cons** | **POS** | **NEG** | **cons** | **POS** | **NED** | **cons** |
| LDT MBR | 0 | 6 | **G** | 0 | 3 | **G** | 0 | 3 | **G** |
| LDT MBR3' | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| LDT mcr | 0 | 3 | **G** | 0 | 1 | **I** | 0 | 2 | **I** |
| Biomed-2 Tube A | 0 | 4 | **G** | 0 | 3 | **G** | 0 | 3 | **G** |
| Biomed-2 Tube B | 0 | 4 | **G** | 0 | 3 | **G** | 0 | 3 | **G** |
| Biomed-2 Tube C | 1 | 3 | **I** | 0 | 3 | **G** | 0 | 3 | **G** |
| IVS Mix1b | 0 | 1 | **I** | 0 | 0 | | 0 | 0 | |
| IVS Mix2b | 0 | 0 | | 0 | 0 | | 0 | 0 | |

**Table 7: Summary of mutation assay results including polymorphisms**

| | | L/L 2013-04 | | L/L 2013-05 | | L/L 2013-06 | |
|---|---|---|---|---|---|---|---|
| **Gene** | **exons/codons tested** | **Result (WT if not indicated)** | **# of variants detected** | **Result (WT if not indicated)** | **# of variants detected** | **Result (WT if not indicated)** | **# of variants detected** |
| **JAK2 Exon 12** | | | | | | | |
| **JAK2 Exon 13** | | | | | | | |
| **JAK2 exon 14** | codon 617 | | | | 1 | | |
| **MPL** | codon 515 | | | | | | |
| | codon 505/515 | | | | | | |
| | amino acid 490 to 520 region | | | | | c.1489G>A (p.A497T) | 1 |
| | W515L/K | | | | | | |
| | S505, W515 | | | | | | |
| | exon 10 | | | | | c.1489G>A; p.A497T | 1 |
| **FLT3 TKD** | D835 | | | | | | |
| | Exon 20 | | 1 | | | | |
| **CEBPA** | Entire coding region, 1 exon. | | | | | | |
| | exon 1 | | | | | | |
| | exon 1 | | | | | | |
| | all coding | | | | | | |
| | chromosome 19, single exon | | | | | | |
| **IDH1** | | | | | | | |
| **IDH2** | | | | | | | |
| **KIT** | exon 17 | p. N822K (c. 2466T>A) | 1 | | | | |
| | Exons 8, 9, 11, 13, 17 | p. N822K (c. 2466T>A) | 2 | | | | |
| | exon 8/17 | p. N822K (c. 2466T>A) | 2 | | | | |
| | D816; exons 8 & 17 | p. N822K (c. 2466T>A) | 1 | | | | |
| **TP53** | exon 5-9 | g.18331G>A; p.R248Q | 1 | g.18348_18349delinsGA p.I254D | 1 | g. [18731G>T (;) 18749G>A] p.[R267L (;) | 1 |
| | Exons 4, 5, 6, 7, 8, 9 | R248Q; c. 743G>A (p. R248Q) | 2 | I254D; 14087-14088 AT>GA (I254D); c. AT760-761GA (p. I254N) | 3 | c.743G>A (p. R248Q); c.800G>T (p.R267L); c.818G>A (p.R273H) | 2 |
| | exon 2-11 | c.743G>A; p.R248Q | 1 | c.760_761delinsGA; p.I254D | 1 | c.743G>A (p.R248Q); c.800G>T (p.R267L); c.818G>A (p.R273H); polymorphism: c.215C>G (p.P72R) | 1 |
| **KRAS** | codon 12/13/61 | | | | | | |
| | exon 1, 2 | | | | | | |
| | codon 12/13 | | | | | | |
| **NRAS** | codons 12/13/61 | | | | | | |
| | exon 1,2 | | | | | | |
| | exons 2-3 | | | | | | |
| **HRAS** | codons 12/13/61 | | | | | | |
| | exon 1,2 | | | | | | |
| **BRAF** | codon 599-602, exon 15 | | | | | | 1 |
| | codon 600 | | | | | | |
| | exon 11, 12, 15 | | | | | | |
| | Exons 11,12,15, codon V600 | | | | | | |
| | V600E ONLY | | | | | | |
| **EGFR** | exon 19/858 | | | | | | |
| | Exon 19 del | | | | | | |
| | L858 | | | | | | |
| **PIK3CA** | Exons 1,9,20 | | | | | E545D | 3 |
| **PDGFRA** | Exons 12, 18 | | | | | | |
| **WT1** | exons 7 & 9 | | | | | | |
| | exons 7 & 9 | | | | | | |
| **MYD88** | codon 265 | | | | | | |
| **NOTCH1** | exon 34 | | | | | | |
| **RUNX1** | exon 1-8 | | | | | | |

**NOTE** **For each gene the area analyzed is listed with the number of labs reporting varients.**
**No entry in the result columns means no specific mutation data were reported.**

=

Table 8: Summary of methods and reagents used

| | Total | SB | PCR | Seq | PCR + Seq | Lab developed | IVS (Biomed-2) | IVS (not Biomed-2) | Lab developed and IVS (Biomed-2) | Lab developed and IVS (not Biomed-2) | Qualitative | Quantitative |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IGH | 28 | 0 | 28 | 0 | 0 | 8 | 10 | 8 | 1 | 0 | | |
| IGK | 16 | 0 | 16 | 0 | 0 | 4 | 11 | 0 | 0 | 0 | | |
| TRB | 15 | 0 | 15 | 0 | 0 | 2 | 12 | 0 | 0 | 0 | | |
| TRG | 26 | 0 | 26 | 0 | 0 | 12 | 10 | 1 | 0 | 0 | | |
| IGHV | 12 | 0 | 3 | 5 | 4 | 8 | 0 | 0 | 0 | 0 | | |
| IGH/BCL2 | 13 | 0 | 13 | 0 | 0 | 7 | 6 | 0 | 0 | | | |
| IGH/CCND1 | 7 | 0 | 7 | 0 | 0 | 6 | 1 | 0 | 0 | | | |
| IGH/MYC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |

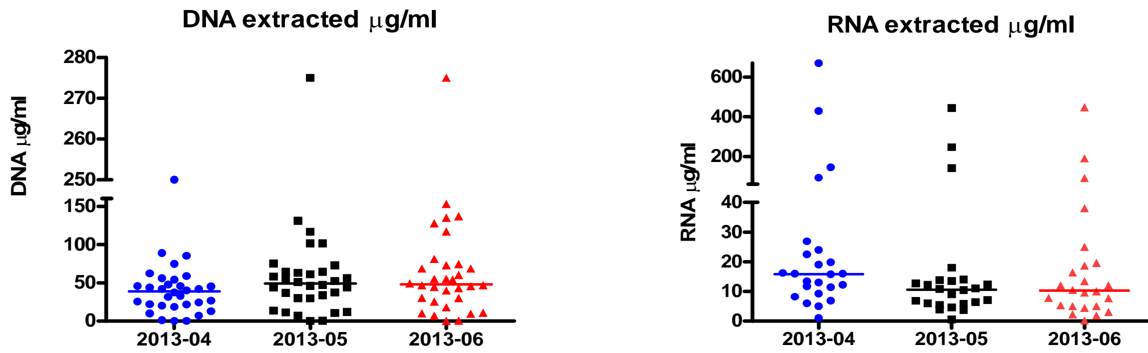
| | Total | PCR | RT-PCR | Seq | PCR + Seq | RT-PCR Seq | Lab developed | Ipsogen (Qiagen) | Seegene | Qualitative | Quantitative | Qual and Quant |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JAK2 V617F | 30 | 23 | 1 | 2 | 1 | 1 | 22 | 6 | 0 | 18 | 7 | 5 |
| JAK2 Exon 12 | 9 | 2 | 0 | 5 | 0 | 0 | 9 | 0 | 0 | | | |
| MPL | 11 | 3 | 1 | 6 | 0 | 0 | 11 | 0 | 0 | | | |
| FLT3 ITD | 8 | 8 | 0 | 0 | 0 | 0 | 7 | 1 | 0 | | | |
| FLT3 TKD | 7 | 6 | 0 | 1 | 0 | 0 | 6 | 1 | 0 | | | |
| NPM1 | 15 | 15 | 0 | 0 | 0 | 0 | 14 | 1 | 0 | | | |
| CEBPA | 7 | 2 | 0 | 4 | 1 | 0 | 7 | 0 | 0 | | | |
| IDH1 | 6 | 2 | 0 | 3 | 1 | 0 | 6 | 0 | 0 | | | |
| IDH2 | 4 | 1 | 0 | 3 | 0 | 0 | 4 | 0 | 0 | | | |
| KIT | 8 | 3 | 0 | 3 | 2 | 0 | 8 | 0 | 0 | | | |

| | Total | PCR | RT-PCR | Seq | PCR + Seq | RT-PCR Seq | Lab developed | Ipsogen (Qiagen) | Roche | Cepheid | Asuragen | Qualitative | Quantitative | Qual and Quant | IS Normalized |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BCR/ABL1 p210 | 28 | 0 | 28 | 0 | 0 | 0 | 18 | 5 | 0 | 2 | 1 | 1 | 23 | 4 | 17 |
| BCR/ABL1 p190 | 26 | 0 | 26 | 0 | 0 | 0 | 18 | 4 | 0 | 0 | 1 | 5 | 17 | 4 | 0 |
| BCR/ABL1 p210/p190 | 5 | 0 | 5 | 0 | 0 | 0 | 4 | 0 | 1 | 0 | 0 | 2 | 3 | 0 | 2 |
| Abl Kinase domain | 7 | 0 | 2 | 2 | 1 | 2 | 7 | 0 | 0 | 0 | 0 | | | | |
| PML/RARA | 13 | 0 | 13 | 0 | 0 | 0 | 12 | 1 | 0 | 0 | 0 | 4 | 9 | 0 | 0 |
| AML1/ETO | 8 | 0 | 8 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 4 | 3 | 0 | 0 |
| NPM1/ALK | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ETV6/RUNX1 | 3 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 |
| CBFB/MYH11 | 4 | 0 | 4 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 |
| TCF3/PBX1 | 1 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| MLL/AF4 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | Total | PCR | Seq | PCR + Seq | Lab developed | Qiagen | Roche Cobas | Assuragen/ Luminex | Sequenom/ Massspec | Other |
|---|---|---|---|---|---|---|---|---|---|---|
| TP53 | 5 | 0 | 4 | 1 | 5 | 0 | | | | |
| KRAS | 10 | 3 | 3 | 4 | 6 | 0 | | | | |
| NRAS | 5 | 0 | 3 | 2 | 4 | 0 | | | | |
| HRAS | 3 | 0 | 1 | 2 | 3 | 0 | | | | |
| BRAF | 11 | 6 | 2 | 3 | 7 | 0 | | | | |
| EBV | 5 | 5 | 0 | 0 | 4 | 0 | | | | 1 |

NOTE: any discrepancies between the numbers in this table and the number of results in Table 1 are caused by incomplete and/or inconsistent data submission by some labs

**Figure 1. NYS MCTM PT 10-2013 DNA and RNA yields. The yields were converted to ug DNA and RNA per 1 ml blood.**




| | 2013-04 | 2013-05 | 2013-06 | | 2013-04 | 2013-05 | 2013-06 |
|---|---|---|---|---|---|---|---|
| | DNA | DNA | DNA | | RNA | RNA | RNA |
| Mean | 43.0 | 56.3 | 62.2 | Mean | 67.1 | 42.4 | 39.8 |
| Median | 38.6 | 49.2 | 48.0 | Median | 15.87 | 10.59 | 10.24 |
| Min | 0.02 | 0.02 | 0.2 | Min | 1.0 | 0.5 | 0.2 |
| Max | 250.0 | 275.0 | 275.0 | Max | 670 | 443 | 448 |