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# Molecular and Cellular Tumor Marker Proficiency Test Event

## MCTM 3-2015

## Summary of results[1]

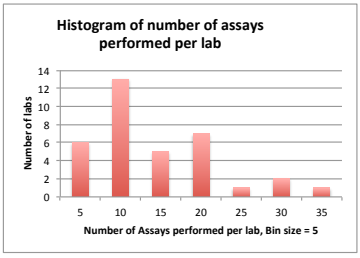
May 11, 2015

Dear Laboratory Director,

Below is a summary and discussion of the New York State Molecular and Cellular Tumor Markers proficiency test event MCTM 3-2015 from March 17, 2015, due date April 15, 2015.

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 between 1 and 33 assays per sample in various combinations. Just over one third of the labs performed between 6 and 10 different assays.




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 only the all method consensus is shown. If there were distinct method specific discrepancies these are discussed in the relevant section below.

[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

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 genotypic marker (**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 or only 2 samples produced a consensus, 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 by your lab 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 2015-01 (Table 1)**

B-cell tests: For IGH and IGK, there was unanimous agreement that these genes were not rearranged. Only one (9%) lab reported a fusion between IGH and BCL2 and no lab reported a fusion between IGH and CCND1. In conclusion, these results suggest that this sample did not contain a clonal B-cell population.

T-cell tests: 13 out of 15 labs (87%) that tested for TRB found a rearrangement, and all 27 labs that tested for TRG reported a rearrangement. The two labs that did not detect the TRB rearrangement should reexamine their results. Interestingly, although the overall results were essentially unanimous, there was some heterogeneity when results were compared by individual primer mixes (tables 4, 5). Together, these results suggest that this sample contained a clonal T-cell population with T-cell receptor gamma and beta gene rearrangements.

Translocations: No translocations/fusions were detected at any of the loci tested.

Various mutations (Table 8): Multiple mutations in presumptive cancer genes were detected. These include a 9 bp deletion in **CALR**, either reported as c.1177_1185del 9 or c.1191_1199del9 or 1120del9 (Table 8 shows the different variations in nomenclature as they were reported). Presumably these all refer to the same 9 bp deletion. Interestingly, four labs (25%) did not find this deletion.

Eight labs found two **TP53** mutations, namely c.524G>A, p.R175H and c.743G>A, p.R248Q, although one lab identified the second mutation as p.R155Q, which may be a data entry error since amino acid 155 in TP53 is a threonine and not an arginine.

Ten labs also found a **KRAS** mutation in codon 12, c.35G>A, p.G12D, and four of seven (57%) labs also reported finding a codon 12 mutation in **NRAS**, c.35G>T, p.G12V. Like for CALR three labs did not find this mutation.

Other mutations found by one or two labs are c.1040G>A, p.R347Q in **EZH2**, c.1193G>A, p.G398D in **ASXL1**, and c.1879G>A, p.A627T in **FLT3**. Furthermore, two of six labs reported a FLT3 IDT mutation.

EBV and other viruses: No lab reported the presence of EBV DNA or any of the other viruses tested for (HTLV-1, HHV8, KSHV).

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a T-cell clone with TRB and TRG rearrangements and multiple mutations in known or suspected cancer genes.

**NYS#L/L 2015-02 (Table 1)**

B-cell tests: For IGH and IGK, there was consensus agreement that these genes were not rearranged except for two labs that reported an indeterminate result for IGH. Furthermore, no lab reported a fusion gene involving either the IGH/BCL2 or IGH/CCND1 loci. Thus, the overall conclusion is that this sample did not contain a clonal B-cell population with immunoglobulin gene rearrangements.

T-cell tests: For TRB and TRG, there was unanimous agreement that these genes were not rearranged. Thus, the overall conclusion is that this sample did not contain a clonal T-cell population with T-cell receptor gene rearrangements.

Translocations: No translocations/fusions were detected.

Various mutations (Table 8): All but one lab (96.5%) detected the **JAK2** V617F mutation in exon 14, and one additional lab reported it as an exon 12 mutation. The one lab that missed this mutation appears to have switched samples two and three. Furthermore, all nine labs (100%) found the c.398T>A, p.M133K mutation in **TP53.**

EBV and other viruses: No lab reported the presence of EBV DNA or any of the other viruses tested for (HTLV-1, HHV8, KSHV).

In aggregate, these results indicate that the sample contained cells from a myeloproliferative neoplasm with the JAK2 V617F mutation, suggesting polycythemia vera (PV), essential thrombocythemia (ET), or primary myelofibrosis (PMF).

**NYS#L/L 2015-03 (Table 1)**

B-cell tests: For both 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 tubes D and E that target the DH1-6 and 7 regions, respectively, and one IVS FR2 primer in one lab (Table 2). Rearrangements in IGK too were detected with all primers (Table 3). No lab reported a fusion between IGH and BCL2 or CCND1, respectively. The results for IGHV were inconsistent. Two labs reported it to be hypermutated with mutation rates of 21.3% and 23.5%, respectively, and six labs considered it clonal, but not hypermutated with mutation rates ranging from 0% to 1.4%. Of those labs that considered IGHV clonal, six assigned it to group VH6-1, one to VH3-20, and one to VH5-51. Interestingly, two of the three labs that also used the mix 2 primers from IVS switched their assignment with mix 2, one from VH6-1 to VH3-20, and the other from VH3-20 to VH6-1. In conclusion, these results suggest that this sample contained a clonal B-cell population with IGH and IGK gene rearrangements.

T-cell tests: For TRB and TRG, there was unanimous agreement that these genes were not rearranged. Thus, the conclusion is that this sample did not contain a clonal T-cell population with T-cell receptor gene rearrangements.

Translocations: One lab reported detecting the MLL/AF4 fusion product, which corresponds to the t(4;11) translocation, whereas another lab that also tested for this fusion did not find it. No other fusion products were detected.

Various mutations (Table 8): no significant mutations were detected. However, two of three labs reported a c.7298T>C, p.F2433S mutation in **NOTCH1**, but considered it a variant of unknown significance, whereas one lab reported NOTCH1 as unmutated. In addition, several polymorphisms in IDH1, CEBPA, TP53, ASXL1 and CKIT were reported, as shown in Table 8. However one lab pointed out that two of these sequence variants, c.211G>A (p.V71I) and c.315C>T (P.G105G) in **IDH1** are also reported in COSMIC and that the combination of c.211G>A (p.V71I) and c.315C>T (P.G105G) had been described previously in 11/531AML patients (7 pediatric AML and 4 adult AML) (Ho PA et al. Leukemia 2010;24(5):909-913).

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal B-cell population, possibly containing the t(4;11) translocation resulting in the MLL/AF4 fusion gene.

**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 2-7). Furthermore, Table 8 shows a summary of the mutation results, and Table 9 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. 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 eluted the nucleic acid into.

**Next generation sequencing**: if your laboratory performs next generation sequencing we encourage you to use these samples to fulfill your proficiency test requirements for NGS. We included all genes for which a mutation was reported by NGS in Table 8, and identified those results that were derived with NGS. Two laboratories provided a spreadsheet with the results from all their targets in their NGS panel, and a side by side comparison is included in this report (Table 10). We encourage other labs to submit complete NGS results in a spreadsheet, so that the results can be included in the comparison in the future. While we certainly don't have enough data to draw a firm conclusion, we noticed that in three instances NGS results were different from those obtained by single gene assays. While in one case NGS detected a mutation where the single gene assay did not (ASXL1 in sample L/L2015-01), in two other instances NGS did not detect a mutation that was detected by single gene assay (NRAS in L/L2015-01, and NOTCH1 in L/L2015-03).

Finally, we would like remind you 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 518-473-4856 or erasmus.schneider@health.ny.gov. For specific questions about your lab's report please contact Ms. Susanne McHale at (518) 486-5775 or susanne.mchale@health.ny.gov.

The next Molecular and Cellular Tumor Marker PT mail-out in 2015 will be:

| **Mail-out date** | **Due Date** |
| --- | --- |
| October 27, 2014 | November 25, 2014 |

Sincerely,

Erasmus Schneider, Ph.D.
Director, Oncology Section
Clinical Laboratory Reference System

**New York State Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 3-2015**
**Table 1: Summary of results**

| Assay / Sample | L/L 2015-01 | | | | | L/L 2015-02 | | | | | L/L 2015-03 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 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 | | 28 | | | G | | 26 | 2 | | G | 28 | | | | R |
| IGK | | 16 | | | G | | 16 | | | G | 16 | | | | R |
| TRB | 13 | 1 | 1 | | R | | 15 | | | G | | 15 | | | G |
| TRG | 27 | | | | R | | 27 | | | G | | 26 | | | G |
| IGHV | | | 1 | 11 | N | | | 1 | 11 | N | 2 | 6 | 5 | | I |
| | POS | NEG | I | | | POS | NEG | I | | | POS | NEG | I | | |
| IGH/BCL2 | 1 | 9 | | | NEG | | 10 | | | NEG | | 10 | | | NEG |
| IGH/CCND1 | | 6 | | | NEG | | 6 | | | NEG | | 6 | | | NEG |
| | MUT | WT | I | | | MUT | WT | I | | | MUT | WT | I | | |
| JAK2 V617F | | 28 | | | WT | 27 | 1 | | | MUT | 1 | 27 | | | WT |
| JAK2 Exon 12 | | 13 | | | WT | 1 | 12 | | | WT | | 13 | | | WT |
| MPL | | 15 | | | WT | | 15 | | | WT | | 15 | | | WT |
| FLT3 ITD | 2 | 6 | | | I | | 8 | | | WT | | 8 | | | WT |
| FLT3 TKD | | 9 | | | WT | | 9 | | | WT | | 9 | | | WT |
| NPM1 | | 17 | | | WT | | 17 | | | WT | | 17 | | | WT |
| CEBPA | | 10 | | | WT | | 10 | | | WT | | 10 | | | WT |
| IDH1 | | 7 | | | WT | | 7 | | | WT | | 6 | 1 | | WT |
| IDH2 | | 6 | | | WT | | 6 | | | WT | | 6 | | | WT |
| KIT | | 11 | | | WT | | 11 | | | WT | | 11 | | | WT |
| CALR | 12 | 4 | | | I | | 16 | | | WT | | 16 | | | WT |
| MyD88 | | 8 | | | WT | | 8 | | | WT | | 7 | | | WT |
| ASXL1 | 1 | 3 | | | I | | 4 | | | WT | | 4 | | | WT |
| | POS | NEG | I | | | POS | NEG | I | | | POS | NEG | I | | |
| BCR/ABL1 p210 | | 27 | | | NEG | | 28 | | | NEG | | 27 | 1 | | NEG |
| BCR/ABL1 p190 | | 26 | | | NEG | | 25 | 1 | | NEG | | 25 | 1 | | NEG |
| BCR/ABL1 p210/p190 | | 5 | | | NEG | | 5 | | | NEG | | 5 | | | NEG |
| | MUT | WT | I | | | MUT | WT | I | | | MUT | WT | I | | |
| ABL Kinase domain | | 3 | | 4 | WT/N | | 3 | | 4 | WT/N | | 3 | | 4 | WT/N |
| | POS | NEG | I | | | POS | NEG | I | | | POS | NEG | I | | |
| PML/RARA | | 12 | | | NEG | | 12 | | | NEG | | 12 | | | NEG |
| AML1/ETO | | 6 | | | NEG | | 6 | | | NEG | | 6 | | | NEG |
| ETV6/RUNX1 | | 2 | | | I | | 2 | | | I | | 2 | | | I |
| CBFB/MYH11 | | 5 | | | NEG | | 5 | | | NEG | | 5 | | | NEG |
| TCF3/PBX1 | | 1 | | | I | | 1 | | | I | | 1 | | | I |
| MLL/AF4 | | 2 | | | I | | 2 | | | I | 1 | 1 | | | I |
| | MUT | WT | I | | | MUT | WT | I | | | MUT | WT | I | | |
| TP53 | 8 | | 1 | | MUT | 9 | | | | MUT | | 9 | | | WT |
| KRAS | 10 | | | | MUT | | 10 | | | WT | | 10 | | | WT |
| NRAS | 4 | 3 | | | I | | 7 | | | WT | | 7 | | | WT |
| HRAS | | 4 | | | WT | | 4 | | | WT | | 4 | | | WT |
| BRAF | | 13 | | | WT | | 13 | | | WT | | 13 | | | WT |
| EGFR | | 9 | | | WT | | 9 | | | WT | | 9 | | | WT |
| PIK3CA | | 5 | | | WT | | 5 | | | WT | | 5 | | | WT |
| | POS | NEG | I | | | POS | NEG | I | | | POS | NEG | I | | |
| EBV | | 4 | | | NEG | | 4 | | | NEG | | 4 | | | NEG |
| Interpretation: | Clonal population of T-lymphocytes with multiple mutations in known or suspected cancer genes, including TP53 and KRAS. | | | | | Myeloproliferative neoplasm, JAK2 V617F positive, TP53 mutation also present | | | | | Clonal lymphoid process with IgH gene rearrangement, consistent with, but not diagnostic of a B-cell neoplasm. IGHV sequence analysis yielded inconclusive results. The sample was also positive for expression of the MLL-AF4 fusionn transcript subtype e10e4, which is the most common form. | | | | |
| 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 2015-01 | | | L/L 2015-02 | | | L/L 2015-03 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **R** | **G** | **cons** | **R** | **G** | **cons** | **R** | **G** | **cons** |
| LDT FR 1 | 0 | 3 | **G** | 0 | 3 | **G** | 3 | 0 | **R** |
| LDT FR 2 | 0 | 7 | **G** | 0 | 7 | **G** | 7 | 0 | **R** |
| LDT FR 3 | 0 | 9 | **G** | 0 | 8 | **G** | 9 | 0 | **R** |
| Biomed-2 Tube A | 0 | 9 | **G** | 0 | 9 | **G** | 9 | 0 | **R** |
| Biomed-2 Tube B | 0 | 10 | **G** | 0 | 10 | **G** | 10 | 0 | **R** |
| Biomed-2 Tube C | 0 | 10 | **G** | 0 | 10 | **G** | 10 | 0 | **R** |
| Biomed-2 Tube D | 0 | 3 | **G** | 0 | 3 | **G** | 0 | 3 | **G** |
| Biomed-2 Tube E | 0 | 4 | **G** | 0 | 4 | **G** | 0 | 4 | **G** |
| IVS FR 1 | 0 | 5 | **G** | 0 | 5 | **G** | 5 | 0 | **R** |
| IVS FR 2 | 0 | 7 | **G** | 0 | 7 | **G** | 6 | 1 | **R** |
| IVS FR 3 | 0 | 7 | **G** | 0 | 6 | **G** | 7 | 0 | **R** |

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

| | L/L 2015-01 | | | L/L 2015-02 | | | L/L 2015-03 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **R** | **G** | **cons** | **R** | **G** | **cons** | **R** | **G** | **cons** |
| LDT Tube A | 0 | 5 | **G** | 0 | 5 | **G** | 5 | 0 | **R** |
| LDT Tube B | 0 | 5 | **G** | 0 | 5 | **G** | 5 | 0 | **R** |
| Biomed-2 Tube A | 0 | 10 | **G** | 0 | 10 | **G** | 10 | 0 | **R** |
| Biomed-2 Tube B | 0 | 10 | **G** | 0 | 10 | **G** | 10 | 0 | **R** |

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

| | L/L 2015-01 | | | L/L 2015-02 | | | L/L 2015-03 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **R** | **G** | **cons** | **R** | **G** | **cons** | **R** | **G** | **cons** |
| LDT Tube A | 2 | 1 | **I** | 0 | 3 | **G** | 0 | 3 | **G** |
| LDT Tube B | 1 | 2 | **I** | 0 | 3 | **G** | 0 | 3 | **G** |
| Biomed-2 Tube A | 6 | 5 | **I** | 0 | 12 | **G** | 0 | 12 | **G** |
| Biomed-2 Tube B | 7 | 4 | **I** | 0 | 12 | **G** | 0 | 12 | **G** |
| Biomed-2 Tube C | 9 | 0 | **R** | 0 | 10 | **G** | 0 | 10 | **G** |

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

| | L/L 2015-01 | | | L/L 2015-02 | | | L/L 2015-03 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **R** | **G** | **cons** | **R** | **G** | **cons** | **R** | **G** | **cons** |
| LDT Vγ1-8 | 5 | 0 | **R** | 0 | 5 | **G** | 0 | 5 | **G** |
| LDT Vγ9 | 0 | 4 | **G** | 0 | 4 | **G** | 0 | 4 | **G** |
| LDT Vγ10 | 0 | 4 | **G** | 0 | 4 | **G** | 0 | 4 | **G** |
| LDT Vγ11 | 0 | 3 | **G** | 0 | 3 | **G** | 0 | 3 | **G** |
| Biomed-2 Tube A | 11 | 0 | **R** | 0 | 11 | **G** | 0 | 11 | **G** |
| Biomed-2 Tube B | 1 | 10 | **G** | 0 | 11 | **G** | 0 | 11 | **G** |
| IVS Mix 1 | 1 | 0 | **I** | 0 | 1 | **I** | 0 | 1 | **I** |
| IVS Mix 2 | 1 | 0 | **I** | 0 | 1 | **I** | 0 | 1 | **I** |
| IVS v2.0 | 5 | 0 | **R** | 0 | 5 | **G** | 0 | 5 | **G** |

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

| | L/L 2015-01 | | | L/L 2015-02 | | | L/L 2015-03 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **POS** | **NEG** | **cons** | **POS** | **NEG** | **cons** | **POS** | **NEG** | **cons** |
| LDT MBR | 0 | 2 | **I** | 0 | 2 | **I** | 0 | 2 | **I** |
| LDT MBR3' | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| LDT mcr | 0 | 1 | **I** | 0 | 1 | **I** | 0 | 1 | **I** |
| Biomed-2 Tube A | 1 | 2 | **I** | 0 | 3 | **G** | 0 | 3 | **G** |
| Biomed-2 Tube B | 0 | 3 | **G** | 0 | 3 | **G** | 0 | 3 | **G** |
| Biomed-2 Tube C | 0 | 3 | **G** | 0 | 3 | **G** | 0 | 3 | **G** |
| IVS Mix1b | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| IVS Mix2b | 0 | 0 | | 0 | 0 | | 0 | 0 | |

**Table 7: Summary for PML/RARA primer mixes**

| | L/L 2015-01 | | | L/L 2015-02 | | | L/L 2015-03 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **POS** | **NEG** | **cons** | **POS** | **NEG** | **cons** | **POS** | **NEG** | **cons** |
| Long | 0 | 5 | **G** | 0 | 5 | **G** | 0 | 5 | **G** |
| Short | 0 | 5 | **G** | 0 | 5 | **G** | 0 | 5 | **G** |
| Varaible | 0 | 3 | **G** | 0 | 3 | **G** | 0 | 3 | **G** |
| L/S/V not distinguished | 0 | 3 | **G** | 0 | 3 | **G** | 0 | 3 | **G** |

**Table 8: Summary of mutation assay results including polymorphisms**

| | | L/L 2015-01 | | L/L 2015-02 | | L/L 2015-03 | |
|---|---|---|---|---|---|---|---|
| Gene | exons/codons tested | Result (WT if not indicated) | # of labs detecting variant | Result (WT if not indicated) | # of labs detecting variant | Result (WT if not indicated) | # of labs detecting variant |
| JAK2 Exon 12 | | | | | | | |
| JAK2 Exon 13 | | | | | | | |
| JAK2 exon 14 | codon 617 | | | MUT (8.2-88.9%) | 28 (3 NGS) | | |
| MPL | codon 515 | | | | | | |
| | codon 505/515 | | | | | | |
| | amino acids 490 to 520 | | | | | | |
| | W515L/K | | | | | | |
| | S505, W515 | | | | | | |
| | exon 10 | | | | | | |
| FLT3 ITD | | MUT | 2 | | | | |
| FLT3 TKD | D835 | | | | | | |
| | Exons 13-15, 20 | c. 1879G>A 9(p. A627T) | 1 NGS | | | | |
| NPM1 | | | | | | | |
| CEBPA | Entire coding region, 1 exon. | | | | | | |
| | exon 1 | | | | | c.690G>T, p.T230T* | |
| | exon 1 | | | | | | |
| | all coding | | | | | | |
| | chromosome 19, single exon | | | | | | |
| IDH1 | | | | | | c.211G>A, p.V71I; c.315G>A, p.G105G* | |
| IDH2 | | | | | | | |
| KIT | exon 17 | | | | | c.2454G>A, p.K818K (suspected, seems to be at low level)* | |
| | Exons 8, 9, 11, 13, 17 | | | | | | |
| | exon 8/17 | | | | | | |
| | D816; exons 8 & 17 | | | | | | |
| CALR | Exon 9 | c. 1177_1185delGAGGATGAG (p. E393_E395 delinsdel) | 1 NGS | | | | |
| | | p.D397_E399del | 1 | | | | |
| | | c.1191_1200delinsA, p.D397_D400delinsE | 1 | | | | |
| | | c.1191_1199del9;p.D397_D400del | 1 | | | | |
| | | c.1191_1199delTGAGGAGGA | 1 | | | | |
| | | c. 1177_1185delGAGGATGAG ( p. E393_E395 delinsdel) | 1 | | | | |
| | | 9 bp deletion, not specified | 4 | | | | |
| | | 8 bp deletion, not specified | 1 | | | | |
| | | not specified | 1 | | | | |
| | | WT | 4 | | | | |
| MYD88 | codon 265 | | | | | | |
| ASXL1 | exons 12, 13 | c. 1193G>A, p. G398D | 1 NGS | | | c.2513A>G, p.K838R; c.3759T>C, p.S1253S* | |
| | exons 12 or 13 or all ex | WT | 4 | | | | |
| TP53 | exons 1-10 | c. 524G>A, p. R175H; c. 743G>A, p. R248Q | 1 NGS | c. 398T>A, p. M133K | 1 NGS | c.215C>G, p.P72R* | |
| | exons 4-10 | c.524G>A (R175H) and c.743G>A (R248Q) | 2 | c. 398T>A, p. M133K | 2 | | |
| | exons 4-9 | 524G>A; p.Arg175His & 743G>A; p. Arg248Gln | 3 (1 NGS) | c. 398T>A (p. M133K) | 3 (1 NGS) | | |
| | Exons 5, 6, 7, 8, 9 | QNS | 1 | 13077 T>A, M133K | 1 | | |
| | exon 2-11 | c. 524G>A(p. R175H) and c. 743G>A(p. R248Q) | 1 NGS | c. 398T>A(p. M133K) | 1 NGS | | |
| | all exons | R155Q; R175H | 1 NGS | M133K | 1 NGS | | |

| | | L/L 2015-01 | | L/L 2015-02 | | L/L 2015-03 | |
|---|---|---|---|---|---|---|---|
| Gene | exons/codons tested | Result (WT if not indicated) | # of labs detecting variant | Result (WT if not indicated) | # of labs detecting variant | Result (WT if not indicated) | # of labs detecting variant |
| KRAS | codon 12/13/61 | GGT-GAT;G12D | 3 | | | | |
| | exons 2, 3 | c. 35G>A (p. G12D) | 2 (1 NGS) | | | | |
| | codon 12/13 | G12D (c.35G>A) | 4 | | | | |
| | all exons | c.35G>A (p.G12D) | 1 NGS | | | | |
| NRAS | codons 12/13/61 | c. 35G>T (p. G12V) | 2 (1 NGS) | | | | |
| | exon 1,2 | c. 35G>T, p. G12V | 1 NGS | | | | |
| | exons 2, 3 | c. 35G>T (p. G12V) | 1 NGS | | | | |
| | codons 12, 13 61 | WT | 1 | | | | |
| | exons 2, 3 | WT | 1 | | | | |
| | all exons | WT | 1 NGS | | | | |
| HRAS | codons 12/13/61 | | | | | | |
| | exon 2, 3 | | | | | | |
| BRAF | codon 599-602, exon 15 | | | | | | |
| | 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 | | | | | | |
| DNMT3A | exons 7-22 | | | | | | |
| EZH2 | exons 1-19 | C. 1040G>A, p. R347Q | 2 (1 NGS) | | | | |
| NOTCH1 | exon 34 | | | | | c.7298 T>C, p.F2433S** | 1 |
| | codons 2370 - 2555 | | | | | c.7298T>C, p.Phe2433Ser** | 1 |
| | exons 26-28 and 34 | | | | | WT | 1 NGS |
| PDGFRA | Exons 12, 18 | | | | | | |
| PHF6 | exons 1-9 | | | | | | |
| RUNX1 | exon 1-8 | c.167T>C, p.L56S* | 1 | | | | |
| SF3B1 | codons 603 - 790 | | | | | | |
| SF3B1 | exons 13-16 | | | | | | |
| TET2 | exons 1-9 | | | | | | |
| WT1 | exons 7 & 9 | | | | | | |
| | exons 7 & 9 | | | | | | |

**Results in red are from NGS**
**For each gene the area analyzed is listed with the number of labs reporting variants.**
**No entry in the result columns means no specific mutation data were reported.**
***likely a polymorphism, as reported by the lab**
****Variant of unknown significance**

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**Table 9: Summary of methods and reagents used**

| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Total | PCR | RT-PCR | Seq (Sanger) | PCR + Seq (Sanger) | RT-PCR + Seq (Sanger) | Seq (Next Gen) | Lab developed | IVS (Biomed-2) | IVS (not Biomed-2) | IVS TRG 2.0 | IVS Lymphotrack | Lab developed and IVS (Biomed-2) | Lab developed and IVS (not Biomed-2) | Qualitative | Quantitative | 0 | 0 |
| IGH | 28 | 28 | 0 | 0 | 0 | 0 | 0 | 9 | 13 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IGK | 16 | 16 | 0 | 0 | 0 | 0 | 0 | 5 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TRB | 15 | 15 | 0 | 0 | 0 | 0 | 0 | 3 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TRG | 27 | 27 | 0 | 0 | 0 | 0 | 0 | 11 | 9 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IGHV | 13 | 3 | 1 | 7 | 1 | 1 | 0 | 9 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IGH/BCL2 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 5 | 4 | 1 | 0 | 0 | 0 | 0 | 8 | 1 | 0 | 0 |
| IGH/CCND1 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 4 | 2 | 0 | 0 |

| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Total | PCR | RT-PCR | Seq (Sanger) | Seq (Pyro) | Seq (Next Gen) | PCR + Seq (Sanger) | PCR + Seq (Pyro) | PCR + Seq (Next Gen) | RT-PCR + Seq (Sanger) | RT-PCR + Seq (Pyro) | RT-PCR + Seq (Next Gen) | Lab developed | Ipsogen (Qiagen) | Illumina | Qualitative | Quantitative | Qual and Quant |
| JAK2 V617F | 28 | 18 | 2 | 0 | 3 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 23 | 4 | 1 | 17 | 7 | 3 |
| JAK2 Exon 12 | 13 | 4 | 0 | 4 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | | | |
| MPL | 15 | 3 | 0 | 4 | 2 | 4 | 0 | 0 | 0 | 2 | 0 | 0 | 14 | 1 | 0 | | | |
| FLT3 ITD | 8 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 7 | 0 | 1 | | | |
| FLT3 TKD | 9 | 5 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 2 | | | |
| NPM1 | 17 | 12 | 0 | 0 | 1 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 15 | 0 | 2 | | | |
| CEBPA | 10 | 0 | 0 | 6 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 2 | | | |
| IDH1 | 7 | 1 | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 2 | | | |
| IDH2 | 6 | 0 | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 2 | | | |
| KIT | 11 | 1 | 0 | 1 | 1 | 4 | 3 | 1 | 0 | 0 | 0 | 0 | 9 | 0 | 2 | | | |
| CALR | 15 | 7 | 0 | 3 | 0 | 2 | 2 | 0 | 1 | 0 | 0 | 0 | 13 | 0 | 2 | | | |
| MyD88 | 8 | 4 | 0 | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 6 | 0 | 2 | | | |
| ASXL1 | 4 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | | | |
| ABL Kinase domain | 7 | 1 | 0 | 1 | 1 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 6 | 0 | 1 | 0 | 0 | 0 |

| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Total | PCR | RT-PCR | Seq | PCR Seq | RT-PCR Seq | Seq (Next Gen) | Lab developed | Ipsogen (Qiagen) | Roche | Cepheid | Asuragen | Illumina | Qualitative | Quantitative | Qual and Quant | IS Normalized | 0 |
| BCR/ABL1 p210 | 27 | 1 | 26 | 0 | 0 | 0 | 0 | 16 | 8 | 0 | 2 | 1 | 0 | 1 | 21 | 5 | 17 | 0 |
| BCR/ABL1 p190 | 25 | 0 | 25 | 0 | 0 | 0 | 0 | 18 | 6 | 0 | 0 | 1 | 0 | 3 | 18 | 4 | | 0 |
| BCR/ABL1 p210/p190 | 5 | 1 | 4 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 1 | 0 | 1 | 4 | 0 | | 0 |
| PML/RARA | 12 | 1 | 10 | 0 | 0 | 0 | 1 | 10 | 1 | 0 | 0 | 0 | 1 | 4 | 7 | 1 | | 0 |
| AML1/ETO | 6 | 0 | 5 | 0 | 0 | 0 | 1 | 4 | 1 | 0 | 0 | 0 | 1 | 3 | 3 | 0 | | 0 |
| ETV6/RUNX1 | 3 | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | | 0 |
| CBFB/MYH11 | 5 | 0 | 4 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 0 | | 0 |
| TCF3/PBX1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | | 0 |
| MLL/AF4 | 2 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | | 0 |

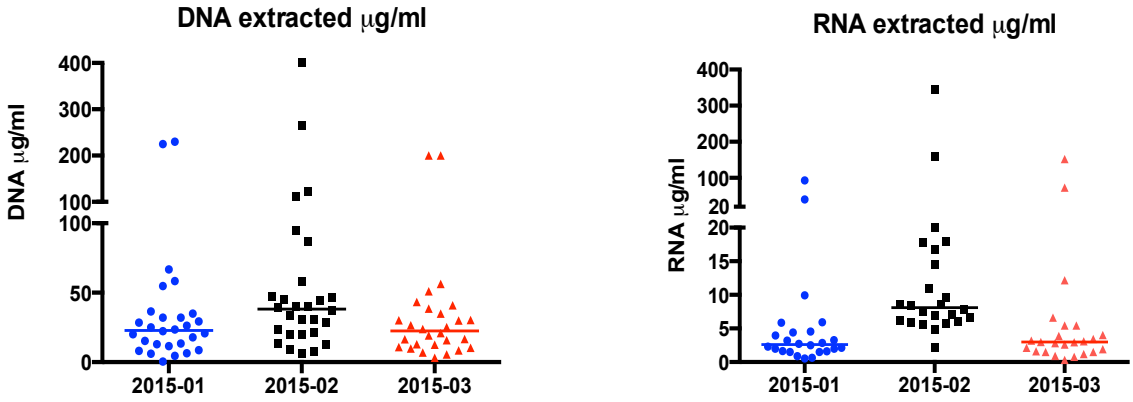
| 0 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 0 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Total | PCR | Seq (Sanger) | Seq (Pyro) | Seq (Next Gen) | PCRSeq (Sanger) | PCRSeq (Pyro) | PCRSeq Next Gen) | Mass Spec | Lab developed | Qiagen | Roche Cobas | Asuragen | Sequenom | Illumina | Other | 0 | 0 |
| TP53 | 9 | 0 | 5 | 0 | 3 | 1 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| KRAS | 9 | 2 | 1 | 2 | 2 | 1 | 1 | 0 | 0 | 5 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 |
| NRAS | 7 | 0 | 1 | 1 | 3 | 1 | 1 | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| HRAS | 4 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| BRAF | 13 | 5 | 0 | 3 | 2 | 2 | 0 | 0 | 1 | 8 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| EGFR | 8 | 2 | 1 | 1 | 2 | 2 | 0 | 0 | 0 | 6 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| PIK3CA | 4 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EBV | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |

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

**Table 10: comparison of results between two NGS panels**

| Lab 1 | Lab 1 | Lab 1 | Lab 1 | Lab 1 | Lab 2 | Lab 2 | Lab 2 | Lab 2 | Lab 2 |
|---|---|---|---|---|---|---|---|---|---|
| Myeloid Panel Tests | EXONS | L/L 2015-01 - Result | L/L 2015-02 - Result | L/L 2015-03 - Result | Myeloid Panel Tests | EXONS | L/L 2015-01 - Result | L/L 2015-02 - Result | L/L 2015-03 - Result |
| ABL | 4-6 | WT | WT | WT | | | | | |
| ASXL | 12 | WT | WT | WT | | | | | |
| ATRX | 8, 10-11 and 17-31 | WT | WT | WT | | | | | |
| BCOR | All | WT | WT | WT | | | | | |
| BCOR1 | All | WT | WT | WT | | | | | |
| BRAF | 15 | WT | WT | WT | | | | | |
| CALR | 9 | c. 1177_1185delGAGGATGAG (p. E393_E395 delinsdel) | WT | WT | | | | | |
| CBL | 8 and 9 | WT | WT | WT | CBL | 7-9 | WT | WT | WT |
| CBLB | 9 and 10 | WT | WT | WT | | | | | |
| CBLC | 9 and 10 | WT | WT | WT | | | | | |
| CDKN2A | All | WT | WT | WT | | | | | |
| CEBPA | All | WT | WT | WT | | | | | |
| CSFR3 | 14-17 | WT | WT | WT | CSF3R | 1-15 | WT | WT | WT |
| CUX1 | All | WT | WT | WT | | | | | |
| DNMT3A | All | WT | WT | WT | DNMT3A | 7-22 | WT | WT | WT |
| ETV6 | All | WT | WT | WT | ETV6 | 1-8 | WT | WT | WT |
| EZH2 | All | WT | WT | WT | EZH2 | 1-19 | C. 1040G>A, p. R347Q | WT | WT |
| FBXW7 | 9, 10 and 11 | WT | WT | WT | | | | | |
| FLT3 | 13-15 and 20 | c. 1879G>A 9(p. A627T) | WT | WT | | | | | |
| GATA1 | 2 | WT | WT | WT | | | | | |
| GATA2 | 2-6 | WT | WT | WT | | | | | |
| GNAS | 8 and 9 | WT | WT | WT | | | | | |
| HRAS | 2 and 3 | WT | WT | WT | | | | | |
| IDH1 | 4 | WT | WT | WT | | | | | |
| IDH2 | 4 | WT | WT | WT | | | | | |
| IKZF1 | All | WT | WT | WT | | | | | |
| JAK2 V617F | 14 | WT | c. 1849G>T(p. V617F) | WT | | | | | |
| JAL2 EXON 12+14 | 12 and 14 | WT | WT | WT | | | | | |
| JAK3 | 13 | WT | WT | WT | | | | | |
| KDM6A | All | WT | WT | WT | | | | | |
| KIT | 2, 8-11, 13 and 17 | WT | WT | WT | | | | | |
| KRAS | 2 and 3 | c. 35G>A (p. G12D) | WT | WT | | | | | |
| MLL | 5-8 | WT | WT | WT | MLL-PTD | | WT | WT | WT |
| MPL | 10 | WT | WT | WT | | | | | |
| MYD88 | 3-5 | WT | WT | WT | | | | | |
| NOTCH1 | 26-28 and 34 | WT | WT | WT | | | | | |
| NPM1 | 12 | WT | WT | WT | | | | | |
| NRAS | 2 and 3 | c. 35G>T (p. G12V) | WT | WT | | | | | |
| PDGFRA | 12, 14 and 18 | WT | WT | WT | | | | | |
| PHF6 | All | WT | WT | WT | PHF6 | 1-9 | WT | WT | WT |
| PTEN | 5 and 7 | WT | WT | WT | | | | | |
| PTPN11 | 3 and 13 | WT | WT | WT | | | | | |
| RAD21 | All | WT | WT | WT | | | | | |
| RUNX1 | All | WT | WT | WT | RUNX1 | 3-8 | WT | WT | WT |
| SETBP1 | 4 | WT | WT | WT | SETBP1 | 3 | WT | WT | WT |
| SF3B1 | 13-16 | WT | WT | WT | SF3B1 | 13-16 | WT | WT | WT |
| SMC1A | 2, 11, 16, 17 | WT | WT | WT | | | | | |
| SMC3 | 10, 13, 19, 23, 25, 28 | WT | WT | WT | | | | | |
| SRSF2 | 1 | WT | WT | WT | SRSF2 | 1 | WT | WT | WT |
| STAG2 | ALL | WT | WT | WT | | | | | |
| TET2 | 3-11 | WT | WT | WT | TET2 | 1-9 | WT | WT | WT |
| TP53 | 2-11 | c. 524G>A(p. R175H) c. 743G>A(p. R248Q) | c. 398T>A(p. M133K) | WT | | | | | |
| U2AF1 | 2 and 6 | WT | WT | WT | U2AF1 | 2,6 | WT | WT | WT |
| WT1 | 7 and 9 | WT | WT | WT | | | | | |
| ZRSR2 | All | WT | WT | WT | ZRSR2 | 2-5, 7-11 | WT | WT | WT |

**Figure 1: NYS MCTM PT 3-2015 DNA and RNA yields. The yields were converted to ug DNA and RNA per 1 ml blood.**




| | L/L 2015-01 | L/L 2015-02 | L/L 2015-03 | | L/L 2015-01 | L/L 2015-02 | L/L 2015-03 |
|---|---|---|---|---|---|---|---|
| | DNA | DNA | DNA | | RNA | RNA | RNA |
| Mean | 38.3 | 62.1 | 35.7 | Mean | 8.6 | 30.4 | 12.7 |
| Median | 22.9 | 38.3 | 22.4 | Median | 2.70 | 7.8 | 3.0 |
| Min | 0.50 | 6.33 | 3.1 | Min | 0.5 | 2.1 | 0.3 |
| Max* | 230.0 | 400.0 | 200.0 | Max | 93 | 344.0 | 152.0 |

*Graph excludes DNA yield from one lab as there clearly was an erroneous number entered