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Molecular and Cellular Tumor Marker Proficiency Test Event

MCTM 10-2015

Summary of results[1]

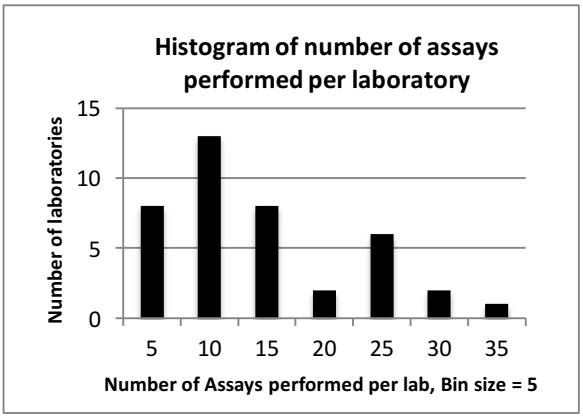
December 14, 2015

Dear Laboratory Director,

Below is a summary and discussion of the New York State Molecular and Cellular Tumor Markers proficiency test event MCTM 10-2015 from October 27, 2015, due date November 25, 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 40 laboratories participated, performing between 1 and 33 assays per sample in various combinations as shown in the figure below. One third of the laboratories 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



[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

less than three laboratories performed a test, or if the concordance between laboratories was less than 80%.

Each laboratory will receive a personalized result sheet by regular mail that shows your laboratory's results in comparison to the all laboratory 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 laboratory 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 laboratory 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 laboratory 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. 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 2015-04 (Table 1)

B-cell tests: For IGH and IGK, there was unanimous agreement that these genes were not rearranged. Similarly, neither a IGH/BCL2 or a IGH/CCND1 fusion were detected. Together, these results suggest that this sample did not contain a clonal B-cell population.

T-cell tests: 16 out of 16 laboratories (100%) that tested for TRB found a rearrangement, and 26/27 laboratories that tested for TRG reported a rearrangement. 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, except for one laboratory that reported a bcr/abl p210 fusion at 4.7%, presumably in error.

Various mutations (Table 8): Multiple mutations in presumptive cancer genes were detected, as described below (a discussion of NGS results follows towards the end).

There was unanimous (12/12) agreement that TP53 was mutated, with all but one laboratory reporting the presence of the four mutations c.524G>A, p.R175H; c.743G>A, p.R248Q; c.844C>T, p.R282W; and c.215C>G, p.P72R, which some laboratories classified as a SNP.

Similarly, 11/12 laboratories also found a **KRAS** mutation in codon 12, c.35G>A, p.G12D.

Finally, 5/9 laboratories also found mutations in PIK3CA, c.211G>A; p.V71I (VOUS); and p.I391M (SNP). However, these were classified as either a variant of unknown significance or a SNP, and thus may not have been reported by some of the other laboratories that tested for this.

A small number of other mutations were found in FLT3, IDH2, EGFR, ASLX1 and RUNX1, as shown in Table 8, but generally by only one or two laboratories each, which in some cases may have been the only laboratories testing for a particular gene.

EBV and other viruses: No laboratory reported the presence of EBV DNA or any of the other viruses tested for.

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, including TP53 and KRAS.

NYS#L/L 2015-05 (Table 1)

B-cell tests: All but one laboratory (28/29) agreed that IGH was rearranged, and 17/17 laboratories also found an IGK rearrangement. Furthermore, there was 100% consensus that there was a IGH/CCND1 but not a IGH/BCL2 fusion. IGHV was generally found to not be hypermutated with mutation rates between 0.01 and 1.68%, but assigned to the class VH1-2. The exception was one laboratory that classified IGHV as VH5-51 and found a 2.4% mutation rate. Thus, the overall conclusion is that this sample did contain a clonal B-cell population with immunoglobulin gene rearrangements and a t(11;14) translocation.

T-cell tests: For TRB and TRG, there was general consensus that these genes were not rearranged with one exception for TRB. 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 other than IGH/CCND1 were detected.

Various mutations (Table 8): Multiple mutations in presumptive cancer genes were detected, as described below (a discussion of NGS results follows towards the end).

There was unanimous (12/12) agreement that TP53 was mutated, with all but one laboratory reporting the presence of the three mutations c.734G>A; p.G245D; c.949C>T; p.Q317*; and c.215C>G, p.P72R, which some laboratories classified as a SNP.

A small number of other mutations were found in IDH1, NOTCH1, ASXL1, SF3B and MET, as shown in Table 8, but generally by only one or two laboratories each, which in some cases may have been the only laboratories testing for a particular gene.

EBV and other viruses: No laboratory reported the presence of EBV DNA or any of the other viruses tested for.

In aggregate, these results indicate that the sample contained clonal B-cells with a IGH/CCND1 fusion, suggesting mantle cell lymphoma.

NYS#L/L 2015-06 (Table 1)

B-cell tests: There was unanimous agreement that IGK was rearranged. In contrast, there was a >96% consensus that IGH was not rearranged in this sample and neither a IGH/BCL2 nor IGH/CCND1 fusion was detected. Thus, these results suggest that this sample contained a clonal B-cell population with an IGK, but not an IGH gene rearrangement.

T-cell tests: For both TRB and TRG, there was unanimous agreement that these genes were not rearranged, suggesting that this sample did not contain a clonal T-cell population.

Translocations: No translocations in any gene tested were detected.

Various mutations (Table 8): Multiple mutations in presumptive cancer genes were detected, as described below (a discussion of NGS results follows towards the end).

There was unanimous (12/12) agreement that KRAS was mutated, with all but one laboratory reporting the presence of the codon 12 mutation c.35G>C, p.G12A. One laboratory instead reported finding a different mutation at that position, namely c.35G>T, p. G12V. This laboratory should reexamine its results for that locus.

No consensus was reached for any of the other mutations detected, including c.853G>A, p.E285K in TP53 (8/12 laboratories), and c.2252C>T; p.T751I in EGFR (4/10 laboratories). A small number of other mutations were found in IDH1, NOTCH1, ASXL1, and JAK3, as shown in Table 8, though some of these were classified as SNP or VOUS and would not necessarily have

been reported. Generally these were only found by one or two laboratories each, which in some cases may have been the only laboratories testing for a particular gene.

EBV and other viruses: No laboratory reported the presence of EBV DNA or any of the other viruses tested for.

In aggregate, these results indicate that the sample contained a clonal B-cell population with only IGK rearranged and a KRAS mutation.

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.

Next generation sequencing: Three laboratories submitted their complete NGS results, though laboratory 3 reported only those for which they found an alteration. In tables 10-12 we aggregated the results for comparison, but only show those genes that were both tested by more than one laboratory and for which at least one laboratory found a mutation. Therefore, for any gene on your panel that is not listed in the tables, your laboratory was either the only one testing for it and thus there is no comparison available, or there was consensus that the gene was wild type. For those genes sequenced by more than one laboratory there was only partial agreement between the laboratories in the mutations detected or whether the gene was mutated at all. There are several possible reasons for this observation. First, since all three laboratories used targeted panels it is possible that there were differences in the actual area of the genes sequenced with each laboratory covering different non-overlapping areas. Second, where more than one laboratory found a mutation, there may be discrepancies in the numbering of the nucleotides/amino acids that could result in the same mutation having a different apparent position. Lastly, since the data were reported in a non-standardized format in some cases a comparison was difficult. For example, laboratory 3 only gave details for mutations that changed the amino acid but did not report the underlying nucleotide change, and for synonymous mutations did not give the corresponding nucleotide change or location.

Finally, **As of January 2016, there will no longer be any New York State proficiency tests offered for the Molecular and Cellular Tumor Marker category.**

However, your laboratory is still required to meet NYS Clinical Laboratory Quality Assessment Sustaining Standard of Practice 3 (QA S3): Ongoing Verification of Examination Accuracy, which requires bi-annual verification of test accuracy. This requirement can be met by, for

example:

1. Enroll in an appropriate PT offered by a CMS-approved provider and authorize the PT provider to release the results to DOH, or
2. Perform an internal bi-annual accuracy verification through re-testing of blinded samples, or parallel testing with another laboratory.

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 laboratory's report please contact Ms. Susanne McHale at (518) 486-5775 or susanne.mchale@health.ny.gov.

Sincerely,

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

New York State Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 10-2015

Table 1: Summary of results

Assay / Sample	L/L 2015-04					L/L 2015-05					L/L 2015-06				
	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		29			G	28	1			R	1	28			G
IGK		17			G	17				R	17				R
TRB	16				R	1	15			G		15			G
TRG	26	1			R		26			G		25	1		G
IGHV				14	N	1	11	1	1	U		1	3	10	I
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
IGH/BCL2		11			NEG		11			NEG		10	1		NEG
IGH/CCND1		7			NEG	7				POS		7			NEG
	MUT	WT	I			MUT	WT	I			MUT	WT	I		
JAK2 V617F		32			WT		32			WT		32			WT
JAK2 Exon 12		13			WT		13			WT		13			WT
MPL		17			WT		17			WT		17			WT
FLT3 ITD		10			WT		10			WT		10			WT
FLT3 TKD	2	10			WT		12			WT		12			WT
NPM1		18			WT		18			WT		18			WT
CEBPA		11			WT		11			WT		11			WT
IDH1		11			WT		11			WT		10	1		WT
IDH2	1	7			WT		8			WT		8			WT
KIT		14			WT		14			WT		14			WT
CALR		19			WT		19			WT		19			WT
MyD88		9			WT		9			WT		9			WT
ASXL1		4			WT	1	1	1		I		3			WT
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
BCR/ABL1 p210	1	30			NEG		31			NEG		31			NEG
BCR/ABL1 p190		29			NEG		28	1		NEG		29			NEG
BCR/ABL1 p210/p190		2			I		2			I		2			I
	MUT	WT	I			MUT	WT	I			MUT	WT	I		
ABL Kinase domain		2		7	I		2		7	I		2		7	I
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
PML/RARA		15			NEG		15			NEG		15			NEG
AML1/ETO		8			NEG		8			NEG		8			NEG
ETV6/RUNX1		3			NEG		3			NEG		3			NEG
CBFB/MYH11		6			NEG		6			NEG		6			NEG
TCF3/PBX1		1			I		1			I		1			I
MLL/AF4		3			NEG		3			NEG		3			NEG
	MUT	WT	I			MUT	WT	I			MUT	WT	I		
TP53	12				MUT	12				MUT	8	4			I
KRAS	11	1			MUT		12			WT	12				MUT
NRAS		10			WT		10			WT		10			WT
HRAS		8			WT		8			WT		8			WT
BRAF		15			WT		15			WT		15			WT
EGFR	1	9			WT		10			WT	4	6			I
PIK3CA	5	4			I		9			WT		9			WT
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
EBV		4			NEG		4			NEG	1	3			I
Interpretation:	Clonal population of T-lymphocytes with multiple mutations in known or suspected cancer genes, including TP53 and KRAS.					B-cell clonal population with Bcl-1 fusion and P53 mutation; possibly Mantle Cell Lymphoma					Clonal B-cell population with IGK rearrangment only and KRAS mutation				
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 2015-04			L/L 2015-05			L/L 2015-06		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT FR 1	0	4	**G**	4	0	**R**	0	4	**G**
LDT FR 2	0	7	**G**	7	0	**R**	0	7	**G**
LDT FR 3	0	9	**G**	8	1	**R**	0	9	**G**
Biomed-2 Tube A	0	10	**G**	9	1	**R**	1	9	**G**
Biomed-2 Tube B	0	11	**G**	10	1	**R**	1	10	**G**
Biomed-2 Tube C	0	11	**G**	10	1	**R**	1	10	**G**
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	6	**G**	6	0	**R**	0	6	**G**
IVS FR 2	0	8	**G**	0	7	**G**	0	8	**G**
IVS FR 3	0	8	**G**	8	0	**R**	0	8	**G**

Table 3: Summary for IGK primer mixes

	L/L 2015-04			L/L 2015-05			L/L 2015-06		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT Tube A	0	4	**G**	4	0	**R**	3	1	**I**
LDT Tube B	0	4	**G**	0	4	**G**	4	0	**R**
Biomed-2 Tube A	0	13	**G**	13	0	**R**	13	0	**R**
Biomed-2 Tube B	0	13	**G**	0	13	**G**	13	0	**R**

Table 4: Summary for TRB primer mixes

	L/L 2015-04			L/L 2015-05			L/L 2015-06		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT Tube A	2	0	**I**	0	2	**I**	0	2	**I**
LDT Tube B	1	1	**I**	0	2	**I**	0	2	**I**
Biomed-2 Tube A	9	4	**I**	0	14	**G**	0	14	**G**
Biomed-2 Tube B	9	5	**I**	0	14	**G**	0	14	**G**
Biomed-2 Tube C	12	0	**R**	1	11	**G**	0	12	**G**

Table 5: Summary for TRG primer mixes

	L/L 2015-04			L/L 2015-05			L/L 2015-06		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT Vγ1-8	5	0	**R**	0	5	**G**	0	5	**G**
LDT Vγ9	1	3	**I**	0	4	**G**	0	4	**G**
LDT Vγ10	2	2	**I**	0	4	**G**	0	4	**G**
LDT Vγ11	1	2	**I**	0	3	**G**	0	3	**G**
Biomed-2 Tube A	12	1	**R**	0	13	**G**	0	13	**G**
Biomed-2 Tube B	2	11	**G**	0	13	**G**	0	12	**G**
IVS Mix 1	2	0	**I**	0	2	**I**	0	2	**I**
IVS Mix 2	2	0	**I**	0	2	**I**	0	2	**I**
IVS v2.0	4	0	**R**	0	4	**G**	0	4	**G**

Table 6: Summary for BCL2 primer mixes

	L/L 2015-04			L/L 2015-05			L/L 2015-06		
	POS	**NEG**	**cons**	**POS**	**NEG**	**cons**	**POS**	**NEG**	**cons**
LDT MBR	0	5	**G**	0	5	**G**	0	5	**G**
LDT MBR3'	0	0		0	0		0	0	
LDT mcr	0	2	**I**	0	2	**I**	0	2	**I**
Biomed-2 Tube A	0	6	**G**	0	6	**G**	0	6	**G**
Biomed-2 Tube B	0	6	**G**	0	6	**G**	0	6	**G**
Biomed-2 Tube C	0	6	**G**	0	6	**G**	0	5	**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 2015-04			L/L 2015-05			L/L 2015-06		
	POS	**NEG**	**cons**	**POS**	**NEG**	**cons**	**POS**	**NEG**	**cons**
Long	0	6	**G**	0	6	**G**	0	6	**G**
Short	0	6	**G**	0	6	**G**	0	6	**G**
Varaible	0	3	**G**	0	3	**G**	0	3	**G**
L/S/V not distinguished	0	1	**I**	0	1	**I**	0	1	**I**

Table 8: Summary of mutation assay results including polymorphisms (as reproted by laboratories)

		L/L 2015-04		L/L 2015-05		L/L 2015-06	
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							
MPL							
FLT3 TKD	D835						
	Exon 20	c.1879G>A; p.A627T	2 NGS				
CEBPA	Entire coding region, 1 exon.					c.690G>T, p.T230T (SNP)	1
IDH1		c.A1239C; pK413N (not reported)	1 NGS	c.315C>T, p.G105G (SNP)	1 NGS	c.211G>A, p.V71I (SNP); c.315C>T, p.G105G (SNP)	1 NGS
IDH2							
KIT							
TP53	illumina The TruSeq Amplicon - Cancer Panel (TSACP)	282 R/W, 248 R/Q, 175 R/H, 72 P/R (SNP)	1 NGS	245 G/D, 72 P/R	1 NGS	285 E/K	1 NGS
	Exons 4-9	c.524G>A; p.Arg175His; c.844C>T; p.Arg282Trp	1	c.734G>A; p.Gly245Asp	1		
	exon 2-11	c.524G>A, p.R175H; c.743G>A, p.R248Q; c.844C>T, p.R282W; c.215C>G, p.P72R (SNP)	10	c.949C>T; p.Q317* c.734G>A; p.G245D; c.215C>G, p.P72R (SNP)	10	c.853G>A, p.E285K	6
KRAS	Codons 12,13,14, 61, 117,146	WT	1			c.35G>T; p.G12V	1
	exons 1-5	c.35G>A; p.G12D	11			c.35G>C; p.G12A	11
NRAS							
HRAS							
BRAF							
EGFR	Exons 1-28	c.3352G>A; p.A1118T	1 NGS			c.2252C>T; p.T751I	1
	Exons 18-21					c.2252C>T, p.Thr751Ile	1
	Targerted Gene Panel					p.T751I	1
	Illumina The TruSeq Amplicon - Cancer Panel (TSACP)					751T/I 5%	1
PIK3CA	Exons 2-11	c.211G>A; p.V71I (VOUS); p.I391M (SNP)	5 (4 NGS)				
PDGFRA							
WT1							
MYD88							
NOTCH1	codons 2370-2555			c.7283delA, p.His2428Profs*7	1	c.7112C>G, p.Thr2371Ser	1
ASXL1	Whole Gene	c.3759T>C, p.S1253S (SNP)		c.2395G>T, p.D799Y (VOUS); c.3029C>T, p.T1010M (VOUS)	2	c.3973C>T, p.L1325F (VOUS); c.3759T>C, p.S1253S (SNP)	1
CALR							
SF3B1	Exon 34			p.H2429fs.	1		
MET				p.E168D	1		
JAK3	Trageted Gene Panel					p.P132T	1 NGS
RUNX1	Exon 1 - 8	c.236T>C, p.V79A (VOUS); c.1108G>A, p.A370T (SNP); c.167T>C, p.L56S (SNP), c.1389C>G,.p.P463P (SNP)	1				

NOTE **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.

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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			
IGH	29	28	0	0	0	0	0	9	13	7	0	0	0	0	0	0			
IGK	17	17	0	0	0	0	0	5	12	0	0	0	0	0	0	0			
TRB	16	16	0	0	0	0	0	2	14	0	0	0	0	0	0	0			
TRG	27	27	0	0	0	0	0	11	11	1	4	0	0	0	0	0			
IGHV	14	2	3	8	1	0	0	11	3	0	0	0	0	0	0	0			
IGH/BCL2	11	11	0	0	0	0	0	6	4	1	0	0	0	0	10	1			
IGH/CCND1	7	7	0	0	0	0	0	6	1	0	0	0	0	0	5	2			

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
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	Life Technologies	Qualitative	Quantitative	Qual and Quant
JAK2 V617F	32	20	2	1	4	2	1	0	0	0	0	0	24	6	1	0	18	8	4
JAK2 Exon 12	13	3	0	6	0	2	1	0	0	0	0	0	13	0	0	0			
MPL	17	2	0	6	3	4	1	0	0	1	0	0	15	1	1	0			
FLT3 ITD	10	9	0	0	0	1	0	0	0	0	0	0	9	0	0	1			
FLT3 TKD	12	6	0	1	0	4	0	0	1	0	0	0	9	0	2	1			
NPM1	19	13	0	0	1	5	0	0	0	0	0	0	16	0	2	1			
CEBPA	11	0	0	6	0	4	1	0	0	0	0	0	9	0	2	0			
IDH1	11	1	0	2	1	7	0	0	0	0	0	0	6	0	3	2			
IDH2	8	0	0	2	0	6	0	0	0	0	0	0	4	0	2	2			
KIT	14	1	0	2	1	7	2	1	0	0	0	0	9	0	3	2			
CALR	19	8	0	3	0	2	4	0	0	0	0	0	19	0	0	0			
MyD88	9	4	0	1	1	3	0	1	0	0	0	0	8	0	1	0			
ASXL1	4	0	0	1	0	3	0	0	0	0	0	0	3	0	1	0			
Abl Kinase domain	9	0	0	4	1	2	1	0	0	1	0	0	9	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
BCR/ABL1 p210	31	2	27	0	0	0	0	19	8	0	2	1	0	4	22	4	20
BCR/ABL1 p190	29	2	25	0	0	0	0	22	6	0	0	1	0	6	20	3	
BCR/ABL1 p210/p190	2	0	2	0	0	0	0	1	0	1	0	0	0	0	2	0	
PML/RARA	15	0	13	0	0	0	1	13	2	0	0	0	0	5	9	1	
AML1/ETO	8	0	7	0	0	0	1	7	2	0	0	0	0	4	4	0	
ETV6/RUNX1	3	0	1	0	0	0	0	2	0	0	0	0	0	1	2	0	
CBFB/MYH11	6	0	4	0	0	0	1	6	0	0	0	0	0	3	3	0	
TCF3/PBX1	1	0	2	0	0	0	1	3	0	0	0	0	0	3	0	0	
MLL/AF4	3	0	2	0	0	0	1	3	0	0	0	0	0	0	0	0	

0	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
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	Life Technologies	Other
TP53	12	0	5	0	6	1	0	0	0	9	0	0	0	0	1	2	0
KRAS	12	2	0	2	4	1	1	0	1	7	1	0	0	1	1	1	1
NRAS	10	0	1	1	5	1	1	0	1	5	1	0	0	0	2	2	0
HRAS	8	0	0	0	5	1	1	0	0	4	0	0	0	0	2	2	0
BRAF	15	4	0	3	4	2	0	0	1	8	3	0	0	1	0	0	3
EGFR	9	1	2	2	3	1	0	0	1	5	1	0	0	0	1	1	0
PIK3CA	8	0	2	0	5	0	0	0	0	3	0	0	0	0	0	0	4
EBV	4	4	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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: NGS comparison for Sample L/L 2015-04

	LAB 1			LAB 2	LAB 3		
Gene	AA Change	MAF	cDNA	AA Change	AA Change	Problems	MAF
ALK	R1209X	0.29	c.C3625T	c.3625C>T, p.R1209*			
APC	C947Y	0.28	c.G2840A	c.2840G>A, p.C947Y	p.A1582P	edge of read	6.4
APC	.				real, synonymous		46.5
ASXL1	K6N	0.31	c.G18C	WT			
ATM	T2059I	0.14	c.C6176T	c.6176C>T, p.T2059I			
BAP1	V603V	0.38	c.C1809T	c.1673G>A, p.S558N			
BCL6	M148T	0.27	c.T443C	c.443T>C, p.M148T			
BRCA1	T1794T	0.32	c.C5382G	c.5318C>A, p.T1773N			
BRCA2	p.I605fs	0.32	c.1813delA	c.1813delA, p.I6054fs*9			
CALR	D59D	0.2	c.C177T	WT			
CD79A	L193L	0.28	c.G579A	WT			
CREBBP	p.K1082fs	0.33	c.3244delA	c.3250delA, p.I1084Sfs*15			
DDR2	V250M	0.31	c.G748A	c.748G>A, p.V250M			
DNMT3A	p.H57fs	0.27	c.171delC	WT			
EGFR	C535C	0.24	c.C1605T	WT			
EP300	R568W	0.28	c.C1702T	c.1702C>T, p.R568W			
ERBB2	S261N	0.13	c.G782A	c.692G>A, p.S231N			
ERBB3	G35D	0.11	c.G104A	c.104G>A, p.G35D			
ETV6	L277V	0.28	c.C829G	c.829C>G, p.L277V			
ETV6	S284A	0.26	c.T850G	c.850T>G, p.S284A			
ETV6	Q289E	0.25	c.C865G	c.865C>G, p.Q289E			
EZH2	R347Q	0.25	c.G1040A	c.1040G>A, p.R347Q			
FAT1	L4201I	0.28	c.C12601A	c.12601C>A, p.L4201I			
FAT1	.			c.4636G>A, p.A1546T			
FBXW7	A685V	0.29	c.C2054T	c.2054C>T, p.A685V			
FBXW7	R465C	0.28	c.C1393T	c.1393C>T, p.R465C			
FBXW7	.			c.585-7_585-5delTTT	p.R385C		28.9
FGFR1	WT		WT	c.*1996C>T			
FGFR2	WT		WT	c.533G>A, p.R178H			
FGFR3	L164L	0.28	c.C490T	WT	real, synonymous		100.0
FGFR4	T405N	0.37	c.C1214A	WT			
FLT3	A627T	0.29	c.G1879A	WT			
FLT3	.	0.32			splice site		62.8
GATA3	A395A	0.3	c.C1185T	WT			
HNF1A	p.G288fs	0.22	c.864delG	WT			
HRAS	A59A	0.29	c.C177T	WT	real, synonymous		29.9
HRAS	.				real, synonymous		57.3
IDH1	K413N	0.27	c.A1239C	WT			
IGF1R	R107H	0.28	c.G320A	c.320G>A, p.R107H			
IGF1R	.			c.1532G>A, p.R511Q			
JAK1	p.E858fs	0.28	c.2573delA	c.2580delA, p.K860Nfs*16			
JAK1	p.A428fs	0.23	c.1283dupC	c.1289dupC , p.L431Vfs*22			
KDR	A573T	0.26	c.G1717A	c.1717G>A, p.A573T			
KRAS	WT		WT	WT	p.G12D		28.4
MED12	T615I	0.4	c.C1844T	WT			
MEF2B	WT		WT	c.259-1G>A, null			
MTOR	L115P	0.3	c.T344C	WT			

Table 10: NGS comparison for Sample L/L 2015-04

NOTCH1	P2064T	0.28	c.C6190A	c.6190C>A, p.P2064T			
NOTCH1	R1991R	0.28	c.C5973T	WT			
NOTCH1	H165N	0.31	c.C493A	WT		real, synonymous	4.2
NOTCH1				c.4780_4781ins36,		large insertion	16.4
NOTCH2	R2053H	0.26	c.G6158A	c.6158G>A, p.R2053H			
PALB2	L303L	0.13	c.C909T	WT			
PBRM1	.	0.31		c.1924+1G>T			
PDGFRA	WT		WT	WT		real, synonymous	71.0
PDGFRA	.			WT		real, synonymous	100.0
PIK3CA	V71I	0.16	c.G211A	WT		p.V71I	17.4
PIK3CA	.					p.I391M	47.0
PIK3CA	.					real, synonymous	6.0
PIK3CD	C147C	0.25	c.C441T	WT			
PIK3R1	p.I82fs	0.24	c.244delA	c.1907delA, p.N636Tfs*26			
RB1	WT		WT	c.608-1delG			
RET	.	0.27		c.1759+33G>A, null		real, synonymous	77.9
ROS1	P198L	0.28	c.C593T	WT			
RUNX1	A370T	0.23	c.G1108A	c.1108G>A, p.A370T			
RUNX1	V79A	0.21	c.T236C	c.236T>C, p.V79A			
SMARCB1	L200L	0.3	c.G600A	c.1118+4C>T		real, synonymous	28.4
SMARCB1	.	0.33					
SMC3	WT		WT	c.2535+2_2535+7delTGTGTA			
SPEN	p.I1052fs	0.29	c.3154delA	c.3154delA, p.I1052Sfs*40			
SPEN	P2378P	0.13	c.C7134T				
SPEN	T2555I	0.3	c.C7664T	c.7664C>T, p.T2555I			
SPOP	H347Q	0.25	c.C1041G	c.1041C>G, p.H347Q			
SRC	P56L	0.23	c.C167T	c.167C>T, p.P56L			
SRSF2	S121G	0.2	c.A361G	C.361A>G, p.S121G			
STAT3	P725S	0.31	c.C2173T	WT			
STAT5B	C688C	0.3	c.C2064T	WT			
STK11	H154H	0.29	c.C462T	c.464+3G>A, null			
TCF3	H307Q	0.13	c.C921G	c.921C>G, p.H307Q			
TET2	T1626A	0.24	c.A4876G	c.4876A>G, p.T1626A			
TNFAIP3	WT		WT	c.983C>T, p.A328V			
TP53	R282W	0.28	c.C844T			p.R282W	35.1
TP53	R248Q	0.3	c.G743A			p.R248Q	34.1
TP53	R175H	0.29	c.G524A			p.R175H	26.8
TP53	.					p.P72R	55.4
TSC1	A567A	0.2	c.G1701A	c.170G>A, p.R57H			
TSC1	.			c.1606g>A, p.A536T			
WT1	P129R	0.33	c.C386G	c.386C>G, p.P129R			
WT1	A99V	0.24	c.C296T	c.296C>T, p.A99V			
WT1	.			c.348C>G, p.Y116*			

Mutations agree
One or two labs Mutated, one lab WT or vice versa
Mutations don't agree

Table 11: NGS comparison for Sample L/L 2015-05

L/L 2015-05 LAB 1				LAB 2	LAB 3	
Gene	AAChange	MAF	cDNA	Result	AA Change	MAF
ALK	p.1432_14	0.27	c.4296_42	WT		
APC	S535F	0.29	c.C1604T	c.1604C>T, p.S535F	real, synonymous	100.0
ASXL1	T1010M	0.24	c.C3029T	WT		
EGFR	WT		WT	WT	real, synonymous	48.0
EZH2	G179G	0.23	c.T537G	WT		
FGFR3	WT		WT	WT	real, synonymous	100.0
FOXO1	T333T	0.26	c.C999T	WT		
GATA2	E391E	0.26	c.A1173G	WT		
HRAS	WT		WT	WT	real, synonymous	21.5
IDH1	WT		WT	WT	real, synonymous	26.2
IDH2	R362W	0.44	c.C1084T	WT		
KDR	WT		WT	WT	p.Q472H	19.3
MCL1	WT		WT	c.146A>G, p.N49S		
MET	E168D	0.2	c.G504T	WT	p.E168D	21.1
MET	.				real, synonymous	54.9
MTOR	L170L	0.34	c.G510C	WT		
MYC	V317V	0.19	c.C951T	WT		
MYD88	WT		WT	c.7283delA, p.H2428Pfs*7		
PDGFRA	.			WT	real, synonymous	100.0
PDGFRA	.				real, synonymous	48.8
PIK3CA	WT		WT	WT	p.I391M	35.6
PTPN1	WT		WT	c.1124T>A, p.L375Q		
RET	L11M	0.41	c.C31A	WT	real, synonymous	80.5
SPEN	A3248P	0.1	c.G9742C	WT		
STAT3	A135A	0.35	c.C405T	WT		
STAT5B	L83L	0.23	c.C247T	WT		
TET3	.	0.15		WT		
TNFRSF14	P3P	0.18	c.T9C	WT		
TP53	Q317X	0.19	c.C949T	WT	p.P72R	21.5
TP53	G245D	0.25	c.G734A		p.G245D	19.5
TSC1	V7V	0.23	c.C21G	WT		
WT1	G98G	0.21	c.C294A	WT		

Mutations agree
One or two labs Mutated, one lab WT or vice versa
Mutations don't agree

Table 12: NGS comparison for Sample L/L 2015-06

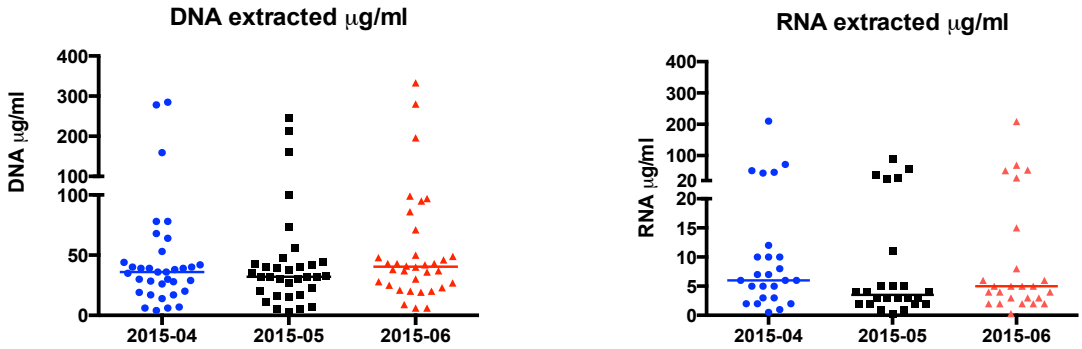
L/L 2015-06	LAB 1			LAB 2	LAB 3		
Gene	**AAChange**	**MAF**	**cDNA**	**Result**	**AA Change**	**MAF**	**Problems**
APC	WT		WT	WT	p.A1582P	5.75	edge of read
APC	.				real, synonymous	59.8	
ATM	WT		WT	WT	real, synonymous	41	
BRAF	WT		WT	WT	real, synonymous	11.1	
CDH1	A817V	0.15	c.C2450T	c.2450C>T, p.A817V			
CREBBP	G2114S	0.31	c.G6340A	c.6340G>A, p.G2114S			
EGFR	C307C	0.39	c.C921T	WT	real, synonymous	52.8	
EGFR	.				p.T751I	5.7	
ERBB4	.	0.34		c.2488-3C>T			
FGFR3	WT		WT	WT	real, synonymous	100	
FGFR4	R437H	0.36	c.G1310A	WT			
HRAS	WT		WT	WT	real, synonymous	97.2	
ID3	WT		WT	c.256G>C, p.E86Q			
IDH1	WT		WT	WT	real, synonymous	10.3	
JAK3	S789L	0.1	c.C2366T	c.2366C>T, p.S789L	p.P132T	13.9	
KDR	WT		WT	WT	p.Q472H	42.6	
KMT2A	WT		WT	c.7983G>C, p.K2661N			
KRAS	G12V	0.13	c.G35T	WT	p.G12A	8.9	
MET	R218R	0.11	c.G654A	WT	real, synonymous	80.7	
MET	.				real, synonymous	8.8	
MTOR	D2485D	0.11	c.C7455T	WT			
NOTCH1	T2371S	0.11	c.C7112G	c.7112C>G, p.T2371S			
PDGFRA	WT		WT	WT	real, synonymous	100	
PDGFRB	H624H	0.37	c.T1872C	WT			
PIK3CA	WT		WT	WT	p.I391M	42.8	
RET	WT		WT	WT	real, synonymous	100	
SF3B1	A1072A	0.35	c.T3216C	WT			
SMARCB1	WT		WT	WT	p.T72K	15.7	edge of read
SPEN	K1064E	0.1	c.A3190G	c.3190A>G, p.K1064E			
TCF3	T508M	0.15	c.C1523T	c.1523C>T, p.T508M			
TP53	E285K	0.15	c.G853A	WT	p.E285K	11.8	
TP53	.				p.F109S	1.3	low freq
TSC2	D1636D	0.31	c.C4908T	WT			
VHL	WT		WT	WT	real, synonymous	3	

Mutations agree

One or two labs Mutated, one lab WT or vice versa

Mutations don't agree

Figure 1: NYS MCTM PT 10-2015 DNA and RNA yields. The yields were converted to ug DNA and RNA per 1 ml blood.



	L/L 2015-04	L/L 2015-05	L/L 2015-06		L/L 2015-04	L/L 2015-05	L/L 2015-06
	DNA	DNA	DNA		RNA	RNA	RNA
Mean	53.0	46.9	62.2	Mean	21.8	11.2	19.4
Median	37.2	31.9	41.5	Median	5.80	3.5	4.7
Min	4.40	2.85	5.8	Min	0.5	0.3	0.3
Max*	285.0	245.0	332.5	Max	210	89.0	209.0

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