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HEALTH

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Molecular and Cellular Tumor Marker Proficiency Test Event
MCTM 10-2014
Summary of results[1]

December 15, 2014

Dear Laboratory Director,

Below is a summary and discussion of the New York State Molecular and Cellular Tumor Markers proficiency test event MCTM 10-2014 from October 28, 2014, with a due date of November 26, 2014.

Samples: All laboratories received three (3) different whole blood 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 36 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 laboratories performed a test, or if the concordance between laboratories 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.

Each laboratory will receive a personalized result sheet by regular mail that shows the 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 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 agreed with the consensus for all 3 samples. 1/2 would mean only 2 samples produced a consensus and you agreed with the consensus for only one of them. The assay score is indicated in the 'score' column to the right of

[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 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 percent '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. However, 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 2014-04 (Table 1)

B-cell tests: For IGH and IGK there was overwhelming agreement that these genes were rearranged, except for one laboratory that did not detect the IGH rearrangement and one laboratory that reported IGH as indeterminate. Rearrangements in IGH were detected with all FR1 and FR2 primers irrespective of their provenance, whereas 5/24 laboratories reported results from their FR3 primers as not rearranged (Table 2). Rearrangements in IGK were, with one exception, only detected with tube B primers that target the Vk-Kde region (Table 3). 10/10 laboratories reported a fusion between IGH and BCL2 in the major breakpoint region (Table 6), whereas no laboratory reported a fusion between IGH and CCND1. Seven of the twelve laboratories (58%) that tested for IGHV detected a hypermutation in family 3-23 with a median mutation rate of 13.9% (range 2.7-15.6%) resulting in an indeterminate result as to whether this sample was hypermutated. In conclusion, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements and the IGH/BCL2 fusion gene, and possibly IGHV hypermutation.

T-cell tests: For TRB and TRG there was overwhelming agreement that these genes were not rearranged, except for one laboratory that reported an indeterminate result for TRG rearrangement (Table 1). The results from all primer mixes were consistent. Together, these results suggest that this sample did not contain a clonal cell population with T-cell receptor beta and/or gamma gene rearrangements.

Translocations: Other than IGH/BCL2 no translocations/fusions were detected at any of the loci tested, except for one laboratory that reported a very low level of the bcr/abl p210 fusion product. This is most likely a false positive result, possibly caused by carry-over or contamination from sample L/L 2014-05 (see below), especially since this laboratory also reported a low level of the bcr/abl fusion product for sample L/L 2014-06. This laboratory should reevaluate their QC procedures.

Various mutations (Table 8): Eight laboratories tested for TP53 mutations, and all eight detected the c.412G>C, p.A138P mutation. In addition, one laboratory also identified a double mutation in PTEN (c.827A>G+829A>G, p.N276S+T277A (same allele)), a double mutation in MLL3 (c.2074_5delGT, p.V629fs; c.12418C>T, p.Q4140X), and a single mutation in EZH2 (c.1936T>A, p.Y646N), all by next generation sequencing.

Viruses: No laboratory reported detecting EBV, HTLV-1, HHV8 and/or KSHV.

The results from all other tests performed were negative/not detected or wild-type.

In aggregate, these results indicate that the sample contained a B-cell clone with IGH and IGK rearrangements and an IGH/BCL2 fusion and possibly IGHV hypermutation, suggesting the presence of B-cell lymphoma cells. The results from Flow Cytometry indicated a mature B cell clone with surface expression of CD10, CD19, CD20, CD21, CD22, CD38, lambda, and HLA-DR, but negative for CD5, CD33, and CD138 (results provided by the cellular immunology section).

NYS#L/L 2014-05 (Table 1)

B-cell tests: For IGH and IGK there was unanimous agreement that these genes were not rearranged. Furthermore, no laboratory reported a fusion 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 overwhelming agreement that these genes were not rearranged, except for one laboratory that reported both TRB and TRG rearrangements and one laboratory that found a TRG rearrangement (Table 1). These laboratories should reevaluate their results, and especially check for contamination. The results from all primer mixes were consistent. Together, these results suggest that this sample did not contain a clonal cell population with T-cell receptor beta and/or gamma gene rearrangements.

Translocations: Twenty seven laboratories detected high levels of the bcr/abl p210 major fusion product, and 13/25 laboratories also detected small amounts (<0.1%) of the bcrl/abl p190 minor fusion product (Figure 1). An additional five laboratories used an assay the does not distinguish between the different breakpoints, but all reported the presence of high levels of a fusion product, in agreement with the consensus from the other laboratories. No other translocations/fusions were detected.

Various mutations (Table 8): Seven out of eight laboratories (87.5%) detected a deletion in TP53, c.697_699delCAC, p.H233del, though there seemed to be some inconsistency in the nomenclature of how it was reported (compare the various entries in Table 8, which are exact copies of what was reported). In addition, one laboratory found a mutation in CEBPA, A240_L241insLA, and two laboratories found two or three, respectively, mutations in ASXL1, c.1934dupG, p.G646fs (found by both laboratories); c.2222A>T, p.D741V (found by both laboratories); c.3692C>T, p.S1231F.

Viruses: No laboratory reported detecting EBV, HTLV-1, HHV8 and/or KSHV.

The results from all other tests performed were negative/not detected or wild-type.

In aggregate, these results indicate that the sample contained bcr/abl positive myeloid cells, presumably CML, with a TP53 mutation. The results from Flow Cytometry indicated megakaryoblast (AML) cells that expressed CD13 and CD33 and were negative for CD2, CD3, CD4, CD5, CD7 CD8, CD10 and CD34; however there was substantial variance with other markers (results provided by the cellular immunology section).

NYS#L/L 2014-06 (Table 1)

B-cell tests: For IGH and IGK, there was unanimous agreement that these genes were rearranged, and with a few exceptions all FR1, 2, and 3 targeting primers detected the rearrangement in IGH (Table 2). Rearrangements in IGK too were detected with all primers (Table 3). Only one laboratory reported a fusion between IGH and BCL2, and no laboratory reported the IGH/CCND1 fusion. Six laboratories reported an unmutated IGHV rearrangement belonging the 3-15 family. In conclusion, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: There was unanimous agreement that this sample contained a TRG rearrangement, and 3/13 laboratories also reported a TRB rearrangement, detected by all three laboratories with the Biomed-2 tube A primers (Table 4). This result is just short (77%) of the 80% needed for a consensus negative call for TRB. Thus, this sample contained clonal cells with TRG rearrangement in addition to the immunoglobulin gene rearrangements.

Translocations: 4/27 laboratories reported a bcr/abl p210 fusion product, albeit at very low levels, raising the question whether these results represent carry-over or contamination from sample L/L 2014-05, which was highly positive for the p210 fusion product. It is recommended that these laboratories reevaluate their QC procedures. Two laboratories detected a ETV6/RUNX1 fusion product. Since these were the only two laboratories that tested for this translocation, we cannot definitively say that this sample contained this fusion product.

Various mutations (Table 8): 3/10 laboratories detected a CEBPA mutation, c.632C>T, p.A211V, and two laboratories reported a p.Q250X mutation in MyD88, with one laboratory commenting that it was not activating and thus not reportable.

Viruses: No laboratory reported detecting EBV, HTLV-1, HHV8 and/or KSHV.

The results from all other tests performed were negative/not detected or wild-type.

In aggregate, these results indicate that the sample contained a B-cell clone with a TRG rearrangement, although from the results it can't be excluded that there were separate B- and T-cell clones. However, the results from Flow Cytometry indicated immature B-cells that expressed surface CD10, CD19, CD38, TdT, and HLA-DR, but were negative for CD5, CD20, CD23, CD11b, CD33, kappa, and lambda (results provided by the cellular immunology section).

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 bcr/abl quantity distribution for sample L/L 2014-05. Figure 2 shows the DNA and RNA yield distributions for the three samples.

Finally, we would like to add some general comments. You really need to follow our instructions for filling out the result form and use the **correct** abbreviations, or we cannot guarantee accurate 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. 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 laboratory 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 laboratory for which you hold or have applied for a NYS permit. Some of you used next generation sequencing to assess the mutation status of the samples. We would encourage others to do the same, which will allow us to make meaningful comparisons and you to meet your PT requirements for this technology. However, per CMS you are not allowed to test for the same mutation by next generation sequencing and with an individual assay until after the event closes.

Some of you reported problems with submitting the result form from the 'Submit' button. Though we are still trying to figure out what the cause for this problem was as not everybody seemed to be affected, we strongly suspect it is connected to the particular combination of operating system and Adobe Reader that your computer has. Please note, Adobe Reader **X** does **not** work, so if you have that particular version we encourage you to upgrade to Adobe Reader **XI** (it is free and can be obtained at http://get.adobe.com/reader). We apologize for the difficulties some of you had and will hopefully identify and rectify the problem before the next PT.

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 or the evaluation please contact 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**
March 17, 2015	April 15, 2015

Sincerely,

Erasmus Schneider, Ph.D.
Director, Oncology Section
Clinical Laboratory Evaluation Program
Wadsworth Center, Room E260
Empire State Plaza
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New York State Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 10-2014
Table 1: Summary of results

Assay / Sample	L/L 2014-04					L/L 2014-05					L/L 2014-06				
	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	24	1	1		R		26			G	26				R
IGK	15				R		15			G	15				R
TRB		13			G	1	12			G	3	10			I
TRG		24	1		G	2	23			G	25				R
IGHV	7			5	I				11	N		7	3	2	I
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
IGH/BCL2	10				POS		10			NEG	1	8	1		NEG
IGH/CCND1		6			NEG		6			NEG		6			NEG
	MUT	WT	I			MUT	WT	I			MUT	WT	I		
JAK2 V617F		29			WT		29			WT		29			WT
JAK2 Exon 12		13			WT		13			WT		13			WT
MPL		15			WT		15			WT		15			WT
FLT3 ITD		8			WT		8			WT		8			WT
FLT3 TKD		8			WT		8			WT		8			WT
NPM1		17			WT		17			WT		17			WT
CEBPA		10			WT	1	9			WT	3	7			I
IDH1		7			WT		6			WT		6			WT
IDH2		6			WT		6			WT		6			WT
KIT		11			WT		11			WT		11			WT
CALR		14			WT		14			WT		14			WT
MyD88		7			WT		7			WT	1	6			WT
ASXL1		4			WT	2	2			I		4			WT
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
BCR/ABL1 p210	1	26			NEG	27				POS	4	23			NEG
BCR/ABL1 p190		26			NEG	13	11	1		I		25	1		NEG
BCR/ABL1 p210/p190		5			NEG	5				POS		5			NEG
	MUT	WT	I			MUT	WT	I			MUT	WT	I		
ABL Kinase domain		1			I		6			WT		1			I
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
PML/RARA		12			NEG		12			NEG		12			NEG
AML1/ETO		7			NEG		7			NEG		7			NEG
ETV6/RUNX1		2			I		2			I	2				I
CBFB/MYH11		4			NEG		4			NEG		4			NEG
TCF3/PBX1															
MLL/AF4		1			I		1			I		1			I
	MUT	WT	I			MUT	WT	I			MUT	WT	I		
TP53	8				MUT	7	1			MUT		8			WT
KRAS		10			WT		10			WT		10			WT
NRAS		7			WT		7			WT		7			WT
HRAS		4			WT		4			WT		4			WT
BRAF		13			WT		13			WT		13			WT
EGFR		6			WT		6			WT		6			WT
PIK3CA		4			WT		4			WT		4			WT
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
EBV		4			NEG		4			NEG		4			NEG
Interpretation:	Clonal B-cell with BCL-2 fusion gene and IGHV hypermutation					bcr/abl MBR (p210) fusion transcript detected, also low level of p190; consistent with CML					Clonal B-cells with IGH, IGK and possibly TRG rearrangement. But results do not allow to distinguish whether the rearrangements are in the same population or in coexisting B and T cell clones.				
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 2014-04			L/L 2014-05			L/L 2014-06		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT FR 1	3	0	**R**	0	3	**G**	3	0	**R**
LDT FR 2	7	0	**R**	0	7	**G**	7	0	**R**
LDT FR 3	3	4	**I**	0	8	**G**	8	0	**R**
Biomed-2 Tube A	11	0	**R**	0	11	**G**	10	1	**R**
Biomed-2 Tube B	11	0	**R**	0	12	**G**	12	0	**R**
Biomed-2 Tube C	11	0	**R**	0	12	**G**	8	4	**I**
Biomed-2 Tube D	1	2	**I**	0	3	**G**	1	2	**I**
Biomed-2 Tube E	0	4	**G**	0	4	**G**	0	4	**G**
IVS FR 1	5	0	**R**	0	5	**G**	5	0	**R**
IVS FR 2	7	0	**R**	0	7	**G**	7	0	**R**
IVS FR 3	5	1	**R**	0	8	**G**	8	0	**R**

Table 3: Summary for IGK primer mixes

	L/L 2014-04			L/L 2014-05			L/L 2014-06		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT Tube A	0	4	**G**	0	4	**G**	4	0	**R**
LDT Tube B	4	0	**R**	0	4	**G**	4	0	**R**
Biomed-2 Tube A	1	10	**G**	0	11	**G**	11	0	**R**
Biomed-2 Tube B	11	0	**R**	0	11	**G**	10	0	**R**

Table 4: Summary for TRB primer mixes

	L/L 2014-04			L/L 2014-05			L/L 2014-06		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT Tube A	0	2	**I**	0	2	**I**	0	2	**I**
LDT Tube B	0	2	**I**	0	2	**I**	0	2	**I**
Biomed-2 Tube A	0	12	**G**	0	10	**G**	3	9	**I**
Biomed-2 Tube B	0	12	**G**	0	11	**G**	0	12	**G**
Biomed-2 Tube C	0	10	**G**	1	8	**G**	0	10	**G**

Table 5: Summary for TRG primer mixes

	L/L 2014-04			L/L 2014-05			L/L 2014-06		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT Vγ1-8	0	6	**G**	1	5	**G**	5	0	**R**
LDT Vγ9	0	5	**G**	0	5	**G**	4	0	**R**
LDT Vγ10	0	5	**G**	0	5	**G**	0	4	**G**
LDT Vγ11	0	3	**G**	0	4	**G**	0	3	**G**
Biomed-2 Tube A	0	11	**G**	1	9	**G**	11	0	**R**
Biomed-2 Tube B	0	11	**G**	0	11	**G**	10	1	**R**
IVS Mix 1	0	1	**I**	0	1	**I**	1	0	**I**
IVS Mix 2	0	1	**I**	0	1	**I**	1	0	**I**
IVS v2.0	0	5	**G**	0	5	**G**	5	0	**R**

Table 6: Summary for BCL2 primer mixes

	L/L 2014-04			L/L 2014-05			L/L 2014-06		
	POS	**NEG**	**cons**	**POS**	**NEG**	**cons**	**POS**	**NEG**	**cons**
LDT MBR	4	0	**POS**	0	4	**NEG**	0	4	**NEG**
LDT MBR3'	0	0		0	0		0	0	
LDT mcr	0	2	**I**	0	2	**I**	0	2	**I**
Biomed-2 Tube A	5	0	**POS**	0	5	**NEG**	1	3	**I**
Biomed-2 Tube B	0	5	**NEG**	0	5	**NEG**	0	5	**NEG**
Biomed-2 Tube C	0	5	**NEG**	0	5	**NEG**	0	5	**NEG**
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 2014-04			L/L 2014-05			L/L 2014-06		
	POS	**NEG**	**cons**	**POS**	**NEG**	**cons**	**POS**	**NEG**	**cons**
Long	0	5	**NEG**	0	5	**NEG**	0	5	**NEG**
Short	0	5	**NEG**	0	5	**NEG**	0	5	**NEG**
Varaible	0	4	**NEG**	0	4	**NEG**	0	4	**NEG**
L/S/V not distinguished	0	2	**I**	0	2	**I**	0	2	**I**

Table 8: Summary of mutation assay results including polymorphisms

		L/L 2014-04		L/L 2014-05		L/L 2014-06	
Gene	exons/codons tested	Result (WT if not indicated)	# of labs	Result (WT if not indicated)	# of labs	Result (WT if not indicated)	# of labs
JAK2	Exon 12						
	Exon 13						
	Codon 617						
MPL	codon 515						
	Codons 505/515						
	Codons 490-520						
	W515L/K						
	S505, W515						
	Exon 10						
FLT3 ITD							
FLT3 TKD	D835						
	Exon 20						
NPM1							
CEBPA	Entire coding region						
	Exon 1					c.632c>T;p.Ala211Val	2
	chromosome 19, single exon			A240_L241 ins LA	1		
	Not indicated					c.632C>T	1
IDH1							
IDH2							
KIT	exon 17						
	Exons 8, 9, 11, 13, 17						
	exon 8/17						
	D816; exons 8 & 17						
CALR	Exon 9						
	Exon 9						
MYD88	Codon 265						
	Exons 3-5					NP_001166038.1: p. Q250*	1
	Whole coding region					Q250X (not activating;not reportable)	1
ASXL1	Exons 12, 13			c1934_1935insG, c. 2222A>T, p. D741V	1		
	Exon 12						
	Ex13 (Some refer it as Ex12)			c.1934dupG ,p.G646fs (COSM34210); c.2222A>T, p.D741V (COSM133580) ; c.3692C>T, p.S1231F	1		
ABL1 kinase domain							
TP53	Exons 4-9	c.412G>C; p.Ala138Pro	2	c.697_699del; p.His233del	1		
	Exons 5-9	c.412G>C; p.Ala138Pro	1	14024-14026 del CAC (del H233)	1		
	Exons 5-9	g.17351G>C; p.A138P	1	g.18284_18286delCCA; p.I232fs	1		
	Exons 1-10	c.412G>C; p.Ala138Pro	1	c. 697_699delCAC	1		
	Exons 2-11	c.412G>C; p.Ala138Pro	1	c.697_699delCAC; p.H233del	1		
	All exons	c.412G>C; p.Ala138Pro	1	c.696_698delCCA; p.H233del	1		
	Not indicated	NP_000537.3: p. A138P	1	NP_000537.3: p.H233del	1		
KRAS	codon 12/13/61						
	exon 1, 2						
	codon 12/13						
NRAS	codons 12/13/61						
	exon 1,2						
	exons 2-3						

		L/L 2014-04		L/L 2014-05		L/L 2014-06	
Gene	exons/codons tested	Result (WT if not indicated)	# of labs	Result (WT if not indicated)	# of labs	Result (WT if not indicated)	# of labs
HRAS	codons 12/13/61						
	exon 1,2						
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						
PDGFRA	Exons 12, 18						
WT1	Exons 7 & 9						
	exons 7 & 9						
NOTCH1	Codons 2370-2555 in exon 34						
RUNX1	Exons 1-8						
PTEN	All exons	c.827A>G+829A>G p.N276S+T277A (same allele)	1				
DNMT3A							
EZH2	All Exons	c.1936T>A; p.Y646N	1				
SF3B1	Codons 603-790						
PHF6	Exons 1-9						
MLL3	All exons	c.2074_5delGT(p.V629fs) + c.12418C>T (Q4140X)	1			c.8950dupT (S2984fs); c.8390delA (K2797fs)	1
MLL-PTD	Exons 2-8						
MLH1	All exons					c.790+1G>A (splice)	1
TET2	Exons 1-9						
CBL	Exons 7-9						

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

Other viruses		L/L 2014-04		L/L 2014-05		L/L 2014-06	
HTLV-1		Neg	2	Neg	2	Neg	2
HHV8		Neg	1	Neg	1	Neg	1
KSHV		Neg	1	Neg	1	Neg	1

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Table 9: Summary of methods and reagents used

	Total	PCR	RT-PCR	Seq	PCR + Seq	RT-PCR + Seq	NGS	Lab developed	IVS (Biomed-2)	IVS (not Biomed-2)	IVS 2.0	IVS Lymphotrack	Qualitative	Quantitative	IMGT V-Quest	NCBI IgBlast
IGH	28	28	0	0	0	0	0	9	12	7	0	0				
IGK	15	15	0	0	0	0	0	5	10	0	0	0				
TRB	15	15	0	0	0	0	0	3	12	0	0	0				
TRG	27	27	0	0	0	0	0	11	10	2	4	0				
IGHV	13	4	2	5	2	0	0	3	0	0	0	0			5	6
IGH/BCL2	10	10	0	0	0	0	0	1	0	0	0	0	9	1		
IGH/CCND1	6	6	0	0	0	0	0	0	0	0	0	0	4	2		

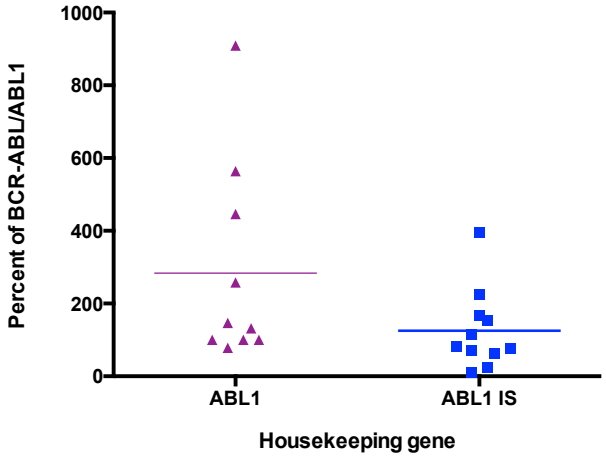
	Total	PCR	RT-PCR	Seq	PCR + Seq	RT-PCR + Seq	NGS	Lab developed	Ipsogen (Qiagen)	Illumina	Qualitative	Quantitative	Qual and Quant
JAK2 V617F	29	18	3	3	2	0	2	23	5	0	18	5	3
JAK2 Exon 12	13	4	1	5	1	0	2	13	0	0			
MPL	15	3	0	7	0	2	3	14	1	0			
FLT3 ITD	8	8	0	0	0	0	0	8	0	0			
FLT3 TKD	8	5	0	1	0	0	2	7	0	1			
NPM1	17	12	0	1	0	0	4	15	0	2			
CEBPA	10	1	0	4	2	0	3	8	0	2			
IDH1	7	1	0	1	1	0	4	5	1	1			
IDH2	6	0	0	1	1	0	4	5	0	1			
KIT	11	1	0	2	4	0	4	10	0	1			
CALR	14	4	0	3	3	0	2	14	0	0			
MyD88	7	2	0	2	1	0	2	6	0	1			
ASXL1	4	0	0	1	0	0	3	2	0	2			

	Total	PCR	RT-PCR	Seq	PCR + Seq	RT-PCR Seq	NGS	Lab developed	Ipsogen (Qiagen)	Roche	Cepheid	Asuragen	Qualitative	Quantitative	Qual and Quant	IS Normalized
BCR/ABL1 p210	29	1	28	0	0	0	0	17	9	0	2	2	1	20	8	22
BCR/ABL1 p190	26	1	25	0	0	0	0	19	6	0	0	1	3	17	6	
BCR/ABL1 p210/p190	5	0	5	0	0	0	0	2	0	2	0	1	1	4	0	
Abl Kinase domain	7	0	1	2	1	2	1	7	0	0	0	0				
PML/RARA	12	0	12	0	0	0	0	11	1	0	0	0	3	8	1	
AML1/ETO	7	0	7	0	0	0	0	6	1	0	0	0	2	5	0	
ETV6/RUNX1	2	0	2	0	0	0	0	2	0	0	0	0	0	2	0	
CBFB/MYH11	4	0	4	0	0	0	0	4	0	0	0	0	2	2	0	
TCF3/PBX1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
MLL/AF4	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	

	Total	PCR	Seq	PCR + Seq	NGS	Mass Spec	Lab developed	Qiagen	Roche Cobas	Assuragen/ Luminex	Sequenom/ Massspec	Illumina	Other
TP53	8	0	4	1	3	0	7	0	0	0	0	1	0
KRAS	10	3	3	2	2	0	6	1	0	1	0	1	1
NRAS	7	0	2	2	3	0	5	1	0	0	0	1	0
HRAS	4	0	0	2	2	0	3	0	0	0	0	1	0
BRAF	13	5	3	2	2	1	6	3	0	1	1	0	2
EGFR	6	1	3	1	1	0	4	1	0	0	0	1	0
PIK3CA	4	0	3	0	1	0	4	0	0	0	0	0	0
EBV	4	4	0	0	0	0	3	1	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 laboratories

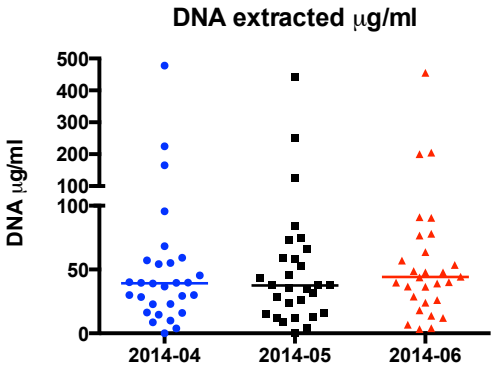
Figure 1: L/L 2014-05 BCR-ABL quantification.

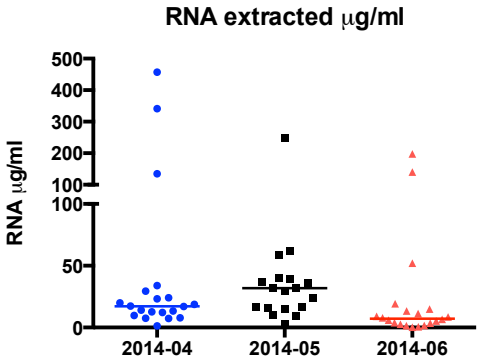


Results of bcr/abl qRT-PCR assays for NYS MCTM L/L 2014-05:

	BCR-ABL/G6PDH			BCR-ABL/TBP			BCR-ABL/BCR			BCR-ABL/ABL1			BCR-ABL/GUSB			BCR-ABL/B2M		
	p210	IS-NCN	p190	p210	IS-NCN	p190	p210	IS-NCN	p190	p210	IS	p190	p210	IS-NCN	p190	p210	IS-NCN	p190
	12%					0.380%		4222%			167%		3.7 ratio			123%		0.013%
	1019%					0.010%				200% of Dx specimen						504%		0.008%
											>10%							
										132%		0.380%						
										100 ratio								
										100%								
											83%							
										100%		0.010%						
											224%	0.010%						
										564%								
											62%							
										78%	23%							
										147%		0.006%						
											115%	0.015%						
											77%							
										258%	395%							
										100%								
											70%							
										909%		0.078%						
										446%	154%	0.032%						
										172 ratio								
Median	515%					0.195%				140%	99%	0.015%				314%		0.011%
Min	12%					0.010%				78%	23%	0.00006				1.2344		0.008%
Max	1019%					0.380%				909%	395%	0.4%				5.0374		0.013%
N	2					2				10	10	7				2		2
Mean	515%					0.195%				283%	137%	0.076%				314%		0.011%
SD	712%					0.262%				275%	108%	0.136%				269%		0.004%

Figure 2: DNA and RNA yields in µg per 1 ml of blood.





	L/L 2014-04	L/L 2014-05	L/L 2014-06		L/L 2014-04	L/L 2014-05	L/L 2014-06
	DNA	DNA	DNA		RNA	RNA	RNA
Mean	61.1	60.4	66.6	Mean	62.3	121.6	26.4
Median	39.0	37.5	43.9	Median	17.00	31.7	7.8
Min	0.07	0.09	3.4	Min	1.3	2.5	0.3
Max*	478.0	442.5	455.0	Max	457.2	1024.9	197.7

*Graph excludes the max RNA yield for L/L 2014-05 because it was far out of line with all other results.