Nirav R. Shah, M.D., M.P.H. Commissioner Sue Kelly Executive Deputy Commissioner

Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 3-2013 Summary of results¹

NEW YORK state department of HEALTH

April 29, 2013

Dear Laboratory Director,

Below is a summary and discussion of the New York State Molecular and Cellular Tumor Markers proficiency test event MCTM 3-2013 from March 19, 2013.

Samples: All laboratories received three (3) different specimens prepared by Wadsworth Center personnel.

Evaluation: Laboratories were asked to perform those molecular assays for which they hold or have applied for a NYS permit. A total of 35 laboratories participated, performing various numbers and combinations of tests. The attached tables summarize the results and methods that were used by participating laboratories. In Table 1, a consensus interpretation is shown of **R**: rearranged/clonal band detected; **G**: germline/no clonal band detected; **WT**: wild-type; **MUT**: mutated; **NEG**: negative or not detected; **POS**: positive or detected; **O**: oligoclonal; **N**: no clonal band or fusion product detected. For IGHV only: **H**: clonal band detected and hypermutated; **U**: clonal band detected, but not hypermutated; **I** (Indeterminate) is shown if no consensus was reached because less than three labs performed a test, or if the concordance between labs was less than 80%. Please note that in a change from previous summary tables, only the all method consensus is shown.

Each lab will receive a personalized result sheet by regular mail that shows your lab's results in comparison to the all lab consensus (if any) derived from all methods combined. Two scores were calculated, one for each assay (**assay score**) across all three samples, and one for each sample (**sample score**) across all assays performed by your lab for each sample. From the latter we also calculated an overall score. Your **assay** score is expressed as a fraction, whereby the denominator is the number of samples you analyzed with a given assay and that were evaluable, i.e. produced a consensus, and the numerator is the number of samples for which you agreed with the consensus. For example, 3/3 means you analyzed all 3 samples and agreed with the consensus for all 3 of them. 1/2 would mean you analyzed only two samples, and 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

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that were evaluable. Assays for which no clear consensus was obtained or for which you were unable to obtain a clear result, as indicated by "I", were not included in either the assay or sample score calculation. At the bottom of each sample column on your result sheet you will find the number of assays performed by your lab for the sample, the number of results that were evaluable and used to calculate the score, and the number of 'correct' answers. The actual sample score as % 'correct' answers was calculated by dividing the number of 'correct' answers by the number of evaluable answers x 100. Finally, we also calculated an overall sample score as the average of the three individual sample scores. At this time we did not assign a grade, but may do so in the future. If any of your results are different from the corresponding consensus we ask that you take a careful look at your analysis and investigate why you may have reported a discrepant result. While this may be because of your assay's design and/or sensitivity and thus does not represent an error *per se*, it could also be a true error, indicating suboptimal performance of your assay, or be due to a contamination in case of apparently false positives.

NYS#L/L 2013-01 (Table 1)

<u>B-cell tests</u>: 20 out of 23 labs (87%) that tested for IGH by PCR reported a rearrangement that was detected by all labs using the Biomed-2 tube B and IVS FR2 primers, and the majority of labs that used LDT FR2 primers (83%, Table 2). Interestingly, a rearrangement was also detected by all nine labs that used the Biomed-2 tube C primers that target the framework 3, but not by the majority of labs that used the original IVS (29%) or LDT FR3 primers (50%). Uniformly, however, none of the FR1 primers detected a rearrangement in this sample (Table 2). One lab also reported result from SB, but found no IGH rearrangement, which was against the PCR consensus results. Eight out of nine labs (89%) reported an IGK rearrangement, possibly in the V κ and J κ regions targeted by the Biomed-2 tube A primers (Table 3). No lab reported a translocation involving the IGH/BCL2 or IGH/CCND1 loci. Nine labs reported IGHV hypermutation that was assigned to the IGHV4-34 family with mutation rates between 12.6-15.4%. These results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements and IGHV hypermutation.

<u>T-cell tests</u>: All 21 laboratories that tested for TRG by PCR found no rearrangement and eleven out of twelve labs (92%) reported no TRB rearrangement by PCR. These results suggest that this sample did not contain cells with T-cell receptor gene rearrangements.

<u>Various mutations (Table 7)</u>: One lab detected a 6bp insertion in the TAD2 region of the CEBPA gene on chromosome 19, which has been described as a polymorphism (c.584_589dup; p.H195_P196dup) with no or undefined clinical relevance, and was also detected by two other labs. One lab detected a p.L265P mutation in MYD88, and one lab detected a heterozygous silent polymorphism in WT1, c.1107A>G, p.R369R. No other mutations were detected in any gene.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal B-cell population with IGH and IGK rearrangements, and IGVH hypermutation. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of mature B-cells expressing CD19/CD20/CD25/kappa.

NYS#L/L 2013-02 (Table 1)

B-cell tests: For IGH and IGK, there was unanimous agreement that these genes were rearranged. Rearrangements in IGH were detected with the Biomed-2 tubes B, C, and D that target the framework 2, 3 and DH 1-6 regions, respectively, but not with the tubes A and E that target the framework 1 and DH7 regions, respectively. Similarly, labs that used the IVS (not Biomed-2) or LDT primers detected rearrangements in the framework 2 and 3, but not framework 1 regions (Table 2). However, neither of the framework 3 targeting primer mixes reached an 80% consensus. Rearrangements in IGK were detected with the Biomed-2 tube B and LDT tube B primers that target the Jk-Ck intron and Kde regions (Table 3). No lab reported a translocation involving the IGH/BCL2 or IGH/CCND1 loci. Of the ten labs that tested for IGHV hypermutation, six reported hypermutation (60%) and assigned it to the IGHV3-21 family with a mutation rate ranging from 11 to 30.6%. In contrast, three labs reported "no clonal band detected" and one lab reported an indeterminate result. Thus, these results suggest that there may be some issues with the assay sensitivity of the labs that did not find a clonal band, but there seemed to be no correlation with whether the assay was LDT- or kit-based. In conclusion, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements and possibly IGHV hypermutation.

<u>T-cell tests</u>: All laboratories that tested for TRB or TRG found no rearrangement. These results suggest that this sample did not contain cell clones with TRB and/or TRG gene rearrangements.

<u>Various mutations (Table 7)</u>: Four labs detected a c.700T>C mutation in TP53 corresponding to p.Y234H, and one of these labs also detected a c.215C>G, p.P72R polymorphism. Furthermore, two labs found a c.690G>T, p.T230T polymorphism in CEBPA, and one lab found a c.303G>T, p.V101V polymorphism in RUNX1. No other mutations were detected in any gene.

EBV: Three out of four labs (75%) reported the presence of EBV sequences.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal B-cell population with a TP53 mutation, and possibly IGHV hypermutation and EBV. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of Burkitt-like B-cells expressing CD19/CD20/ CD23/CD38.

NYS#L/L 2013-03 (Table 1)

<u>B-cell tests</u>: No lab reported any rearrangements or translocations. Thus, there was a consensus that this sample did not contain cell clones with immunoglobulin gene rearrangements or translocations involving the IGH locus.

<u>T-cell tests</u>: All laboratories that tested for TRB or TRG found no rearrangement. These results suggest that this sample did not contain cell clones with T-cell receptor gene rearrangements.

<u>JAK2 V617F</u>: 29 out of 30 labs (97%) reported this mutation with the rate ranging from 7.1 to 76.6%. the one lab that reported WT for this sample reported MUT for sample L/L 2013-02, which is against the consensus in both cases, suggesting a data entry mistake. One lab reported the same c.1849G>T mutation for JAK2 exon 12, which is inconsistent with current JAK2 exon nomenclature that places this mutation in exon 14.

<u>Various mutations (Table 7)</u>: No other mutations were detected; however, one lab reported polymorphisms in IDH1, c.315C>T, p.G105G; TP53, c.215C>G, p.P72R, and WT1, c.1107A>G, p.R369R.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a myeloid cell population with a JAK2 V617F mutation. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of myeloid like MO-myeloblastic (early development) cells expressing CD33/CD34/DR/CD11b/CD11c/CD117.

General comments

The attached tables show summaries of the results both overall (Table 1) as well as for each individual primer mix for the B- and T-cell tests (Tables 2-6). Furthermore, Table 7 shows a summary of the mutation results, and Tables 8 shows summaries of the methods and reagents used for most of the tests. Figure 1 shows the DNA and RNA yield distributions for the three samples. Again the differences in yield are large ranging from 150- to over 1200-fold, raising the question whether everybody reported their results the same way. Please make sure that you report the DNA and RNA yields in microgram (μ g) and based on the actual volume of the original blood sample from which you isolated the DNA and RNA. Do not report the volume as the volume in which you eluded the nucleic acid into. We realize that shipping the samples at room temperature is suboptimal for subsequent RNA analysis. However, because of the combined shipping with the malignant immunophenotyping samples we cannot change that.

Finally, we would like to add some general comments. You really need to follow our instructions for filling out the result form, or we cannot guarantee correct evaluation of your results. You **must select the overall result in the first column**, as it is this result that is used in the evaluation. Then fill in or select the part of the additional information as appropriate. Also please make sure that you choose the correct method where there is a choice. If your starting material is DNA you must choose PCR. If your starting material is RNA, you must choose RT-PCR. Please note: RT stands for **R**everse **T**ranscription, not real time, and thus should only be used for assays whose starting material is RNA. Furthermore, we ask that if you obtain your primers/kits from InVivoScribe you correctly identify the source as IVS (not Biomed-2) (identified as gene rearrangement assays in their catalog) or IVS (Biomed-2) (identified as gene clonality assays in their catalog); for the purpose of this PT evaluation they are not considered lab developed even if you obtain the individual primer tubes separately as ASR reagents instead of as part of a RUO

kit. This will make it easier to compare the performance of individual primer mixes. <u>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.</u>

If you have any questions, comments or suggestions, you may contact me by phone or email at 518-474-2088 or <u>schneid@wadsworth.org</u>. For specific questions about your lab's report or the evaluation please contact Dr. Rong Yao at (518) 474-1744 or <u>yaor@wadsworth.org</u> or Ms. Susanne McHale at (518) 486-5775 or <u>smchale@wadsworth.org</u>.

Please note there was a change to two Molecular and Cellular Tumor Marker PT mail-outs in 2012, with the next one being:

Mail-out date October 22, 2013 Due Date November 20, 2013

Sincerely,

Pelmerdes

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

New York State Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 3-2013
Table 1: Summary of results

Assay / Sample		L/L 2	2013-01				L	/L 2013-0	02		L/L 2013-03					
	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	20	3	1		R	23		1		R		23	1		G	
IGK	8	1			R	9				R		9			G	
TRB		11	1		G		12			G		11			G	
TRG		21			G		21			G		20	1		G	
IGHV	9				н	6		1	3	Т				10	N	
	POS	NEG	I			POS	NEG	I			POS	NEG	I			
IGH/BCL2		10			NEG		10			NEG		10			NEG	
IGH/CCND1		5			NEG		5			NEG		5			NEG	
IGH/MYC																
	MUT*	WT*	I			MUT*	WT*	I			MUT*	WT*	I			
JAK2 V617F		30			WT	1	29			WT	29	1			MUT	
JAK2 Exon 12		9			WT		9			wт	1	8			WT	
MPL		10			WT		10			wт		10			WT	
FLT3 ITD		7			WT		7			WT		7			WT	
FLT3 TKD		6			WT		6			WT		6			WT	
NPM1		12			WT		12			WT		12			WT	
CEBPA		6			WT		6			WT		6			WT	
IDH1		5			WT		5			WT		5			WT	
IDH2		3			WT		3			WT		3			WT	
KIT		7			WT		7			WT		7			WT	
					VVI	500				WI	500				VVI	
	POS	NEG	1			POS	NEG	I			POS	NEG	I			
BCR/ABL1 p210		27			NEG	2	25			NEG		27			NEG	
BCR/ABL1 p190		26			NEG		26			NEG		26			NEG	
BCR/ABL1 p210/p190		9			NEG		8			NEG		8			NEG	
	MUT*	WT*	1	N		MUT*	WT*	I	N		MUT*	WT*	I	N		
ABL Kinase domain		1		6	I		1		6	1		1		6	1	
	POS	NEG	I			POS	NEG	1			POS	NEG	I			
PML/RARA Long		7			NEG		6			NEG		6			NEG	
PML/RARA short		7			NEG		6			NEG		6			NEG	
PML/RARA variable		2			I.		2			I		2			I	
PML/RARA L/S/V																
AML1/ETO		6			NEG		6			NEG		6			NEG	
NPM1/ALK																
ETV6/RUNX1		2			I		2			I		2			I.	
CBFB/MYH11		3			NEG		3			NEG		3			NEG	
TCF3/PBX1		1			I		1			I		1			I	
MLL/AF4		2			I.		2			1		2			1	
	MUT*	WT*	I			MUT	wт	I			MUT*	WT*	I			
ТР53		4			WT	4				MUT		4			wт	
KRAS		10			ωт		10			wт		10			νт	
NRAS		4			νт		4			wт		4			₩Т	
HRAS		2			I.		2			I		2			I	
BRAF		9		1	ωт		9			WT		9			ωт	
	POS	NEG	I			POS	NEG	I			POS	NEG	I			
EBV		4			NEG	3	1			I	1	3			1	
Interpretation:	B-cell with IGF	IV hypermutat	ion	1		B-cell, po	ssibly with and EBV p	IGHV hyp	ermutatior	n; with p53	Myeloprol	iferative n	eoplasm,	JAK2 V617	'F positive	
Comments						The IGHV	hypermutation of r	tion was no	ot consisten	tly	-		Myeloproliferative neoplasm, JAK2 V617F positive			

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

	I	L/L 2013-01			L/L 2013-02	2	L/L 2013-03			
	R	G	cons	R	G	cons	R	G	cons	
LDT FR 1	0	1	I	0	1		0	1	I	
LDT FR 2	5	1	R	6		R	0	6	G	
LDT FR 3	4	4	1	5	3	1	0	8	G	
Biomed-2 Tube A	0	8	G	0	8	G	0	8	G	
Biomed-2 Tube B	10		R	8		R	0	9	G	
Biomed-2 Tube C	9		R	7	2	1	0	8	G	
Biomed-2 Tube D	1	3	1	3		R	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	0	4	G	
IVS FR 2	6		R	5	1	R	0	5	G	
IVS FR 3	2	5	I	3	3	1	0	6	G	

Table 3. Summary for IGK primer mixes

	L/L 2013-01				L/L 2013-02	2	L/L 2013-03			
	R	G	cons	R	G	cons	R	G	cons	
LDT Tube A	2	0	I	0	3	G	0	2	I	
LDT Tube B	0	2	I	3		R	0	2	I	
Biomed-2 Tube A	7	1	R	1	6	G	0	8	G	
Biomed-2 Tube B	0	8	G	7	0	R	1	7	G	

Table 4. Summary for TRB primer mixes

	L/L 2013-01				L/L 2013-02	2	L/L 2013-03		
	R	G	cons	R	G	cons	R	G	cons
LDT Tube A	0	1	I	0	1		0	1	I
LDT Tube B	0	1	I	0	1	1	0	1	I
Biomed-2 Tube A	0	9	G	0	9	G	0	9	G
Biomed-2 Tube B	0	9	G	0	9	G	0	9	G
Biomed-2 Tube C	1	6	G	0	8	G	0	8	G

Table 5. Summary for TRG primer mixes

		L/L 2013-01			L/L 2013-02	2		L/L 2013-03		
	R	G	cons	R	G	cons	R	G	cons	
LDT Vy1-8	0	8	G	0	8	G	0	8	G	
LDT Vy9	0	7	G	0	7	G	0	7	G	
LDT Vy10	0	7	G	0	7	G	0	7	G	
LDT Vy11	0	6	G	0	6	G	0	6	G	
Biomed-2 Tube A	0	10	G	0	10	G	0	10	G	
Biomed-2 Tube B	0	10	G	0	10	G	0	9	G	
IVS Mix 1	0	2	I	0	2	1	0	2	1	
IVS Mix 2	0	2	I	0	2	1	0	2	1	
IVS v2.0	0			0			0			

Table 6. Summary for BCL2 primer mixes

		L/L 2013-01			L/L 2013-02	-	L/L 2013-03		
	POS	NEG	cons	POS	NEG	cons	POS	NED	cons
LDT MBR	0	5	G	0	5	G	0	5	G
LDT MBR3'				0			0		
LDT mcr		3	G	0	3	G	0	3	G
Biomed-2 Tube A		3	G	0	3	G	0	3	G
Biomed-2 Tube B		3	G	0	3	G	0	3	G
Biomed-2 Tube C		3	G	0	3	G	0	3	G
IVS Mix1b				0			0		
IVS Mix2b	0	0		0	0		0	0	

		L/L 2013-01		L/L 2013-02		L/L 2013-03		
Gene	exons/codons tested	Result (WT if not indicated)	# of results entered	Result (WT if not indicated)	# of results entered	Result (WT if not indicated)	# of results entered	
JAK2 Exon 12			9	Silent Mutation c.1929T>C	9		9	
JAK2 Exon 13			1		1		1	
JAK2 exon 14	codon 617		30	MUT, p.V617F	1	MUT, p.V617F	29	
MPL	codon 515		2		2		2	
	codon 505/515		1		1		1	
	exon 10, 11		1		1		1	
	W515L/K		1		1		1	
	S505, W515		1		1		1	
	exon 10		1		1		1	
FLT3 TKD	D835		3		3		3	
	D835/836		3		3		3	
СЕВРА	Entire coding region, 1 exon.	polymorphism: c.584_589dupACCCGC	1	polymorphism: c.690G>T (rs34529039)	1		1	
	exon 1		1		1		1	
	exon 1	polymorphism: c.584_589dup; p.H195_P196dup	1	polymorphism: c.690G>T; p.T230T	1			
	all coding		1		1		1	
	chromosome 19, single exon	polymorphism: 6bp insertion TAD2	1		1		1	
IDH1						polymorphism: c.315C>T; p.G105G	1	
IDH2								
кіт	exon 17		1		1			
	Exons 8, 9, 11, 13, 17		1		1		1	
	exon 8/17		2		2		1	
	D816; exons 8 & 17		1		1		1	
ТР53	exon 5-9		2	p.Y234H	1		2	
	Exons 4, 5, 6, 7, 8, 9		1	14027 T>C (Y234H)	1		1	
	exon 2-11	polymorphism: c.215C>G; p.P72R	1	c.700T>C; p.Y234H polymorphism: c.215C>G; p.P72R	1	polymorphism: c.215C>G; p.P72R	1	
	exon 5-9			g.18288T>C, p.Y234H	1			

Table 7: Summary of mutation assay results including polymorphisms (all results are WT except where indicated)

		L/L 2013-01		L/L 2013-02		L/L 2013-03	
Gene	exons/codons tested	Result (WT if not indicated)	# of results entered	Result (WT if not indicated)	# of results entered	Result (WT if not indicated)	# of results entered
KRAS	codon 12/13/61		3		3		3
	exon 1, 2		1		1		1
	codon 12/13		6		6		6
NRAS	codons 12/13/61		2		2		2
	exon 1,2		1		1		1
	exons 2-3		1		1		1
HRAS	codons 12/13/61		1		1		1
	exon 1,2		1				1
BRAF	codon 599-602, exon 15		1		1		1
	codon 600		5		6		6
	exon 11, 12, 15		1		1		1
	Exons 11,12,15, codon V600		1		1		1
	V600E ONLY		1				
EGFR	exon 19/858		1		1		1
	Exon 19 del		1		1		1
	L858		1		1		1
РІКЗСА	Exons 1,9,20		2		2		2
PDGFRA	Exons 12, 18		1		1		1
WT1	exons 7 & 9		1		1		1
	exons 7 & 9	polymorphism: c.1107A>G; p.R369R	1			polymorphism: c.1107A>G; p.R369R	1
MYD88	codon 265	p.L265P	1		1		1
NOTCH1	exon 34		1		1		1
RUNX1	exon 1-8		1	polymorphism: c.303G>T; p.V101V	1		1

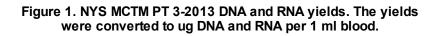
NOTE

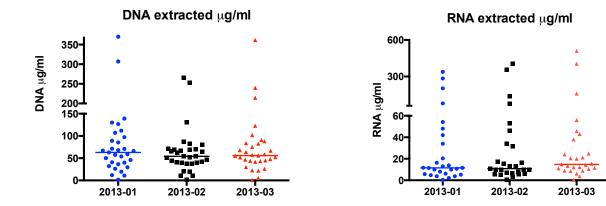
For each gene the area analyzed is listed with the number of labs analyzing the same region No entry in the result columns means no specific mutation data were reported.

Table 8: Summary of methods and reagents used

	Total	SB	PCR	Seq	PCR + Seq	Lab developed	IVS (Biomed- 2)	IVS (not Biomed-2)	Lab developed and IVS (Biomed-2)	Lab developed and IVS (not Biomed-2)	Qualitative	Quantitative			
IGH	26	1	24	0	0	8	9	5	1	1					
IGK	11	0	11	0	0	2	9	0	0	0					
TRB	12	2	11	0	0	3	8	0	0	0					
TRG	22	0	22	0	0	11	10	0	0	0					
IGHV	9	0	1	0	8	6	0	0	0	0					
IGH/BCL2	11	0	11	0	0	6	5	0	0						
IGH/CCND1	5	0	5	0	0	4	1	0	0						
IGH/MYC	0	0	0	0	0	0	0	0	0						
	Total	PCR	RT-PCR	Seq	PCR + Seq	RT-PCR Seq	Lab developed	lpsogen (Qiagen)	Seegene	Qualitative	Quantitative	Qual and Quant			
JAK2 V617F	30	23	1	0	3	0	21	7	0	14	10	5			
JAK2 Exon 12	9	3	0	0	4	0	8	0	0		-	-			
MPL	10	3	1	0	6	0	10	0	0						
FLT3 ITD	7	7	0	0	0	0	6	1	0						
FLT3 TKD	6	6	0	0	0	0	5	1	0						
NPM1	12	12	0	0	0	0	11	1	0						
CEBPA	6	2	0	0	4	0	6	0	0						
IDH1	5	1	0	0	4	0	5	0	0						
IDH2	3	0	0	0	3	0	3	0	0						
KIT	7	2	0	0	5	0	7	0	0						
KI	,	2	Ū	Ū	5	0	,	0	0						
							Lab	Ipsogen						Qual and	
								.poogen							IS Normalized
	Total	PCR	RT-PCR	Sea	PCR + Sea	RT-PCR Sea	developed	(Qiagen)	Roche	Cepheid	Asuragen	Qualitative	Quantitative	Quant	is inormalized
BCR/ABL1 p210	Total 27	PCR 0		Seq 0	PCR + Seq 0	RT-PCR Seq	developed 17	(Qiagen) 3	Roche 0	Cepheid 1	Asuragen 1		and the second	Quant 4	
BCR/ABL1 p210 BCR/ABL1 p190	27	PCR 0 0	RT-PCR 25 23	Seq 0 0	•	RT-PCR Seq 0 0	developed 17 19	(Qiagen) 3 3		Cepheid 1 0	Asuragen 1 1	1	21	Quant 4 4	12 0
BCR/ABL1 p190	27 26	0 0	25	0	0	0	17	3	0 0	1 0	1	1 3	21 18	4 4	12
BCR/ABL1 p190 BCR/ABL1 p210/p190	27	0	25 23	0	0	0	17 19	3 3	0	1	1 1	1	21	4	12 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain	27 26 8	0 0 0	25 23 7	0 0 0	0 0 0	0 0 0	17 19 4	3 3 0	0 0 3	1 0 0	1 1 0	1 3 2	21 18 5	4 4	12 0
BCR/ABL1 p190 BCR/ABL1 p210/p190	27 26 8 7	0 0 0	25 23 7 2	0 0 0 2	0 0 0 0	0 0 0 3	17 19 4 6	3 3 0	0 0 3 0	1 0 0 0	1 1 0 0	1 3	21 18	4 4 0	12 0 1
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA	27 26 8 7 11	0 0 0 0	25 23 7 2 11	0 0 0 2 0	0 0 0 0	0 0 0 3 0	17 19 4 6 10	3 3 0 1	0 0 3 0	1 0 0 0 0	1 1 0 0	1 3 2 3	21 18 5 8	4 4 0	12 0 1 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK	27 26 8 7 11 6	0 0 0 0 0	25 23 7 2 11 6	0 0 2 0 0	0 0 0 0 0	0 0 3 0 0	17 19 4 6 10 6	3 3 0 1 0	0 0 3 0 0	1 0 0 0 0 0	1 1 0 0 0	1 3 2 3 3	21 18 5 8 3	4 4 0 0	12 0 1 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1	27 26 8 7 11 6 0	0 0 0 0 0 0	25 23 7 2 11 6 0	0 0 2 0 0 0	0 0 0 0 0 0 0	0 0 3 0 0 0	17 19 4 6 10 6 0	3 0 0 1 0 0	0 3 0 0 0	1 0 0 0 0 0 0 0	1 1 0 0 0 0	1 3 2 3 3 0 1	21 18 5 8 3 0	4 4 0 0 0	12 0 1 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1 CBFB/MYH11	27 26 8 7 11 6 0 2 3		25 23 7 2 11 6 0 2 3	0 0 2 0 0 0 0 0 0		0 0 3 0 0 0 0 0	17 19 4 6 10 6 0 2 3	3 3 0 1 0 0 0 0	0 3 0 0 0 0 0	1 0 0 0 0 0 0 0 0	1 1 0 0 0 0 0 0 0	1 3 2 3 3 0 1 2	21 18 5 8 3 0 1 1	4 4 0 0 0 0 0 0	12 0 1 0 0 0 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1	27 26 8 7 11 6 0 2	0 0 0 0 0 0 0	25 23 7 2 11 6 0 2	0 0 2 0 0 0 0	0 0 0 0 0 0 0	0 0 3 0 0 0 0	17 19 4 6 10 6 0 2	3 3 0 1 0 0	0 3 0 0 0 0	1 0 0 0 0 0 0 0	1 0 0 0 0 0 0 0	1 3 2 3 3 0 1	21 18 5 8 3 0 1	4 4 0 0 0 0 0	12 0 1 0 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1 CBFB/MYH11 TCF3/PBX1	27 26 8 7 11 6 0 2 3 1		25 23 7 2 11 6 0 2 3 1	0 0 2 0 0 0 0 0 0 0 0 0		0 0 3 0 0 0 0 0 0	17 19 4 6 10 6 0 2 3 1	3 3 0 1 0 0 0 0 0	0 3 0 0 0 0 0 0 0	1 0 0 0 0 0 0 0 0 0 0 0	1 1 0 0 0 0 0 0 0 0 0 0 0	1 3 2 3 0 1 2 1	21 18 5 8 3 0 1 1 1 0	4 4 0 0 0 0 0 0 0	12 0 1 0 0 0 0 0 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1 CBFB/MYH11 TCF3/PBX1	27 26 8 7 11 6 0 2 3 1 2		25 23 7 2 11 6 0 2 3 1 2 3	0 0 2 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 0 0	0 0 3 0 0 0 0 0 0 0	17 19 4 6 10 6 0 2 3 1 2	3 3 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 0 0 0 0 0 0 0 0 0 0 0	1 1 0 0 0 0 0 0 0 0 0 0 0	1 3 2 3 0 1 2 1	21 18 5 8 3 0 1 1 1 0	4 4 0 0 0 0 0 0 0	12 0 1 0 0 0 0 0 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1 CBFB/MYH11 TCF3/PBX1 MLL/AF4	27 26 8 7 11 6 0 2 3 1 2 2 Total	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	25 23 7 2 11 6 0 2 3 1 2 3 5 2 Seq	0 0 2 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 0 0 0 0 0 0 0 0 0 0 0	0 0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	17 19 4 6 10 6 0 2 3 1	3 3 0 1 0 0 0 0 0 0 0	0 3 0 0 0 0 0 0 0 0	1 0 0 0 0 0 0 0 0 0 0 0	1 1 0 0 0 0 0 0 0 0 0 0 0	1 3 2 3 0 1 2 1	21 18 5 8 3 0 1 1 1 0	4 4 0 0 0 0 0 0 0	12 0 1 0 0 0 0 0 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1 CBFB/MYH11 TCF3/PBX1 MLL/AF4 TP53	27 26 8 7 11 6 0 2 3 1 2 3 1 2 Total 4	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	25 23 7 2 11 6 0 2 3 1 2 3 1 2 5eq 0	0 0 2 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 0 0 0 0 0 0 0 0 0	0 0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	17 19 4 6 10 6 0 2 3 1 2	3 3 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 1 0 0 0 0 0 0 0 0 0 0 0	1 3 2 3 0 1 2 1	21 18 5 8 3 0 1 1 1 0	4 4 0 0 0 0 0 0 0	12 0 1 0 0 0 0 0 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1 CBFB/MYH11 TCF3/PBX1 MLL/AF4 TP53 KRAS	27 26 8 7 11 6 0 2 3 1 2 3 1 2 Total 4 10	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	25 23 7 2 11 6 0 2 3 1 2 3 1 2 Seq 0 0	0 0 2 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 0 0 0 0 0 0 0 0 0 0 0	0 0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	17 19 4 6 10 6 0 2 3 1 2	3 3 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 1 0 0 0 0 0 0 0 0 0 0 0	1 3 2 3 0 1 2 1	21 18 5 8 3 0 1 1 1 0	4 4 0 0 0 0 0 0 0	12 0 1 0 0 0 0 0 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1 CBFB/MYH11 TCF3/PBX1 MLL/AF4 TP53 KRAS NRAS	27 26 8 7 11 6 0 2 3 1 2 Total 4 10 4	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	25 23 7 2 11 6 0 2 3 1 2 2 5 eq 0 0 0 0	0 0 2 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 0 0 0 0 0 0 0 0 0 0 0	0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	17 19 4 6 10 6 0 2 3 1 2	3 3 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 1 0 0 0 0 0 0 0 0 0 0 0	1 3 2 3 0 1 2 1	21 18 5 8 3 0 1 1 1 0	4 4 0 0 0 0 0 0 0	12 0 1 0 0 0 0 0 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1 CBFB/MYH11 TCF3/PBX1 MLL/AF4 TP53 KRAS NRAS HRAS	27 26 8 7 11 6 0 2 3 1 2 7 7 0 4 10 4 2	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	25 23 7 2 11 6 0 2 3 1 2 2 5 eq 0 0 0 0 0 0	0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	C C C C C C C C C C C C C C C C C C C	0 0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	17 19 4 6 10 6 0 2 3 1 2 Roche Cobas	3 3 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 1 0 0 0 0 0 0 0 0 0 0 0	1 3 2 3 0 1 2 1	21 18 5 8 3 0 1 1 1 0	4 4 0 0 0 0 0 0 0	12 0 1 0 0 0 0 0 0 0 0
BCR/ABL1 p190 BCR/ABL1 p210/p190 Abl Kinase domain PML/RARA AML1/ETO NPM1/ALK ETV6/RUNX1 CBFB/MYH11 TCF3/PBX1 MLL/AF4 TP53 KRAS NRAS	27 26 8 7 11 6 0 2 3 1 2 Total 4 10 4	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	25 23 7 2 11 6 0 2 3 1 2 2 5 eq 0 0 0 0	0 0 2 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 0 0 0 0 0 0 0 0 0 0 0	0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	17 19 4 6 10 6 0 2 3 1 2	3 3 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 1 0 0 0 0 0 0 0 0 0 0 0	1 3 2 3 0 1 2 1	21 18 5 8 3 0 1 1 1 0	4 4 0 0 0 0 0 0 0	12 0 1 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





	L/L 2013-01	L/L 2013-02	L/L 2013-03		L/L 2013-01	L/L 2013-02	L/L 2013-03
	DNA	DNA	DNA		RNA	RNA	RNA
Mean	80.2	65.9	76.5	Mean	46.4	48.5	56.3
Median	63.0	54.2	56.0	Median	11.60	10.80	14.80
Min	1.6	1.8	1.2	Min	0.4	0.4	0.4
Max	370.0	265.0	361.8	Max	338.1	402.7	510.6