NEW YORK
state department of
HEALTH

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Commissioner

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Executive Deputy Commissioner

Molecular and Cellular Tumor Marker Proficiency Test Program
MCTM 3-11
Summary of results[1]

April 28, 2011

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from March 1, 2011, MCTM 3-11.

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 36 laboratories participated, performing various numbers and combinations of tests. The attached tables summarize the results and methods that were used by participating laboratories. A consensus interpretation of **G** (Germline/normal/wild type) or **R** (Rearranged/mutated/translocated) is also indicated where possible. Please note that **R** includes anything that is not normal, i.e. that is rearranged, translocated, contains a fusion gene or viral sequences, or contains a mutation. Only truly normal samples, i.e. those without any evidence of a disease-related process of any nature, should be called **G** (but see discussion below for TRB for L/L 2). **I** (Indeterminate) is shown if no consensus was reached because less than three labs performed a test, or if the difference between the number of labs reporting R or G is not sufficient to derive a clear consensus, defined as ≥75% agreement between all responses. However, in cases where there was a clear difference in the results obtained with one method vs. another, e.g. southern blot (SB) vs. PCR, then the "consensus" was expressed for each method separately, e.g. **R/G**, where the first letter belongs to the first method, and the second belongs to the second method.

In addition, you also receive a personalized result sheet that gives your lab's result 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. 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. If you reported results from two different methods, each method was scored independently, and the results added together. This 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'

[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

answers per sample (i.e. that agree with the consensus), based on the number of assays performed per sample that were evaluable. Assays, for which no clear consensus was obtained, 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 times 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 apparent false positives.

NYS#L/L 1 (Table 1):
B-cell tests: For IGH, all 30 laboratories reported no rearrangement (28= PCR, 2= SB). Nine out of the ten labs (90%) that tested for IgKappa (IGK) by PCR found no rearrangement; the one lab that reported a rearrangement also was the only one reporting this rearrangement for L/L3. All laboratories that tested for IGH/BCL2 by PCR reported no translocation at any of the three breakpoint clusters. Similarly, none of the five laboratories that tested for the IGH/CCND1 translocation (also known as Bcl-1) by PCR found a rearrangement. Thus, the consensus was that this sample did not contain immunoglobulin gene rearrangements.

T-cell tests: All 27 laboratories that tested for TcRGamma (TRG) by PCR found a rearrangement, possibly in the Vγ1-8 and Vγ10 regions detected by either Biomed-2 tube A, IVS mix 1 and 2, or lab developed Vγ1-8 and Vγ10 primers (Table 6). Likewise, all thirteen labs that tested for TcRBeta (TRB) reported a rearrangement (2=SB, 11=PCR), possibly in the Jβ1 region detected by the Biomed-2 tube A primers (Table 7). These results suggest that this sample contained cells with T-cell receptor beta and gamma gene rearrangements.

P53: One lab out of two (50%) detected a missense mutation (R273C).

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal T-cell population. This conclusion is in agreement with the result from Flow Cytometry, which indicated an immature cell of T-cell lineage.

NYS#L/L 2 (Table 2):
B-cell tests: For IGH, there was unanimous agreement that this locus was rearranged (28=PCR, 1=SB). Rearrangements were detected by PCR using the Biomed-2 tubes A to D that target all three framework regions and six DH regions, whereas results with tube E that targets only the DH 7 region were negative. Similar results were obtained by the labs that used either the original IVS or lab developed primers (Table 4). Nine out of the ten labs that tested for IGK by PCR reported a rearrangement with both Biomed-2 tubes A and B (Table 5). No translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any method. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: 22 out of the 27 laboratories (81%) that tested for TcRGamma (TRG) by PCR found no rearrangement; the five labs that did detect a rearrangement used IVS (2), Biomed-2 (2), or lab developed (1) primers (Table 6). Eight out of the twelve(67%) labs that tested for TcRBeta (TRB) reported no

rearrangement (1=SB, 7=PCR), although two labs detected a rearrangement using the Biomed-2 tube C, but decided to report the overall result as G, since this rearrangement is the incomplete D-J rearrangement and has a poor correlation with the neoplastic process. However, three of the four labs that did report a rearrangement apparently chose to interpret a positive Biomed-2 tube C result as a true rearrangement (Table 7). One laboratory tested for TcRDelta (TRD) by PCR but found no rearrangement. These results suggest that this sample did not contain a T-cell clone, but rather that a small number of the B-cells may also have T-cell receptor rearrangements.

EBV: All three labs that tested for also detected the presence of EBV virus sequences by PCR, which is usually associated with Burkitt lymphoma.

P53: Two labs detected the Y163C mutation.

IGHV mutation: Eight out of the nine labs (89%) that tested for also reported IGHV hypermutation (3=PCR, 5=RT-PCR), of which six assigned it to the IGHV3-11 family, and reported mutation rates between 8.0-9.38%. The rest did not specify the family or the mutation rate.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal B-cell population containing EBV sequences and IGVH hypermutation. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of a B-cell clone.

NYS#L/L 3 (Table 3):
B-cell tests: For IGH, all 28 laboratories reported no rearrangement (27= PCR, 1= SB). Nine out of the ten labs (90%) that tested for IgKappa (IGK) by PCR found no rearrangement; the one lab that reported a rearrangement was also the only one reporting a rearrangement for L/L1 (using the Biomed-2 tube A), though interestingly with the other primer mix (tube B). All laboratories that tested for IGH/BCL2 reported no translocation at any of the three breakpoint clusters. Similarly, none of the five laboratories that tested for the IGH/CCND1 translocation (also known as Bcl-1) found a rearrangement. Thus, the consensus was that this sample did not contain immunoglobulin gene rearrangements.

T-cell tests: All 26 laboratories that tested for TRG by PCR found no rearrangement. Similarly, all eleven labs that tested for TRB found no rearrangement (10=PCR, 1=SB). Therefore, there was a general consensus that this sample did not contain a T-cell clone.

BCR/ABL1: All 26 labs that tested for BCR/ABL1 MBR translocations (25=RT-PCR, 1=PCR) found a fusion gene. Similarly, all seven labs that did not distinguish MBR from mcr by RT-PCR also detected a fusion transcript. Interestingly, 13 out of 22 labs (60%) also detected a BCR/ABL1 mcr fusion gene(12=RT-PCR, 1=PCR), although at a much lower level than the BCR/ABL1 MBR fusion gene (Figure 2).

P53: Two labs reported a mutation; one lab reported detecting a “Q136 fs” mutation, whereas the other did not indicate the type of mutation.

The results from all other tests performed were negative.

Therefore, there was a general consensus that this sample contained cells that express the BCR/ABL1 MBR fusion gene, but there was no consensus as to the presence of the BCR/ABL1 mcr fusion gene. Figure 2 and the associated table show the BCR/ABL1 results from those 26 laboratories that performed

the assay quantitatively. Five different housekeeping genes were used for normalization, and the results were either expressed as a ratio of bcr/abl copies to housekeeping gene copies or as a percentage. The results varied considerably, reflecting the use of different housekeeping genes. Furthermore, even quantitative values that were normalized to the same housekeeping gene, e.g. abl, ranged from 12.67 to 5756%, representing a 454-fold difference, though some labs may have reported the wrong unit, i.e. % instead of ratio or vice versa. Of 26 labs, only three labs indicated that they normalized their results to the international scale, i.e. 118.05%, 100%, and 71.42%, respectively.

These results indicate that the sample contained cells with the t(9;22) translocation expressing the p210 BCR/ABL1 fusion gene. The result from Flow Cytometry indicated a myeloid cell type. Together, the results are consistent with CML.

The attached tables show a summary of the results both in aggregate (Tables 1-3) as well as by individual primer mixes for the B- and T-cell tests (Tables 4-7). Figure 1 shows the DNA and RNA yield distributions for the three samples. DNA yields from samples L/L1, 2, and 3 ranged from a minimum of 5.0, 2.5, and 2.5 µg per 5 ml specimen to a maximum of 13,820, 6,600, and 7,690 µg, respectively, corresponding to a 2,640- to 3,076-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 1.4, 2.1, and 10.1 µg to 4,710, 1,690, and 3,465 µg, respectively, corresponding to a 343- to 3,364-fold difference between lowest and highest yield for each sample. These results raise the question whether everybody reported their results in microgram (µg), or whether some results were reported in nanogram (ng). **Please make sure that you report the DNA and RNA yields in the appropriate volume of the sample, and that your units are in microgram (µg), not nanogram (ng) or milligram (mg).** Possibly, differences in the methods used for DNA and RNA isolation also contributed to this wide range, but it also raises the question of how accurate some of the measurements are. 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. There is sometimes confusion as to where to write the results. 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. If your starting material is DNA you must record your result in the PCR column. Vice versa, if your starting material is RNA, you must report your results in the RT-PCR column. Please make sure that your results are written in the correct column that corresponds to the starting material you used. A few labs did not indicate the methods and/or reagents that they used for their assays. We cannot properly evaluate your results without this information. In particular, we ask that if you obtain your primers from InVivoScribe you correctly identify the source as IVS (identified as gene rearrangement assays in their catalog) or Biomed-2 (identified as gene clonality assays in their catalog); for the purpose of this PT evaluation they are not considered lab developed even if you obtain the individual primer tubes separately as ASR reagents instead of as part of a RUO kit. This will make it easier to compare the performance of individual primer mixes. Finally, we ask that you analyze the samples by all molecular tests performed in your lab for which you hold or have applied for a NYS permit.

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

The dates for the Molecular and Cellular Tumor Marker PT mail-out in 2011 are:

Mail-out date	**Due Date**
June 27	July 26
October 24	November 22

Sincerely,

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

Table 1: New York State Molecular Oncology Proficiency Test

Sample: NYS# L/L1 Consensus Summary 3-11.xls

Interpretation: T-cell clone with TRB and TRG gene rearrangements

Assay	SB				PCR				RT-PCR				All methods			Method used				
	R	G	ind	Cons#	R	G	ind	Cons#	R	G	ind	Cons#	R	G	Cons#	SB	PCR (qualitative)	PCR (quantitative)	RT-PCR (qualitative)	RT-PCR (quantitative)
IGH		2		I		28		G					0	30	G	Dako (1), home brew (1)	Biomed (12), home brew (11), IVS (5)			
IGK					1	9		G					1	9	G		Biomed (9), home brew (1)			
IGL													0	0						
TRB	2			I	11			R					13	0	R	home brew (1), Dako (1)	Biomed (9), home brew (2),			
TRG					27			R					27	0	R		Biomed (11), home brew (13), IVS (3),			
TRD													0	0						
IGH/BCL2 MBR						13		G					0	13	G		Biomed (3), IVS (2), home brew (7	home brew (1)		
mcr						10		G					0	10	G		Biomed (3), IVS (1), home brew (5	home brew (1)		
MBR 3'						3		G					0	3	G		Biomed (3)			
MBR/mcr													0	0						
IGH/CCND1 (Bcl-1)						5		G					0	5	G		home brew (3), IVS (1)	home brew (1)		
BCR/ABL1 p210										24		G	0	24	G				home brew (6)	home brew (16), Ipsogen(4), Cepheid(1
p190										21		G	0	21	G				home brew (8)	home brew (12), Ipsogen(4)
p210/190										11		G	0	11	G				home brew (3)	home brew (3), Roche(4),Asu(1) Ipsogen(1)
Abl kinase domain mutation										2		I	0	2	I				home brew (2)	
PML/RARA Long										13		G	0	13	G				home brew (6)	home brew (6), Ipsogen(1)
Short										12		G	0	12	G				home brew (5)	home brew (6), Ipsogen(1)
Variable										4		G	0	4	G				home brew (3)	Ipsogen(1)
Long/Short/Variable													0	0						
MYC t(8;14)													0	0						
AML1/ETO t(8;21)										5		G	0	5	G				home brew (3)	home brew (2)
NPM/ALK t(2;5)													0	0						
IGH/BCL-6													0	0						
ETV6/RUNX1 (Tel-AML1)										2		I	0	2	I				home brew (1)	home brew (1)
EBV						3		G					0	3	G		home brew (2)	Roche (1)		
KSHV/HHV8						3		G					0	3	G		home brew (3)			
HTLV1						2		I					0	2	I		home brew (2)			
CBFB INV(16)/MYH11										2		I	0	2	I				home brew (1)	home brew (1)
E2A-PBX t(1;19) (4;11)										1		I	0	1	I				home brew (1)	
MLL(11q23)/ AF4 (4;11)										2		I	0	2	I				home brew (2)	
JAK 2 (V617F)						24		G		2		I	0	26	G		home brew (17), Ipsogen(3)	home brew(1) Invader(1) Ipsogen(3)	home brew (2)	home brew (1)
JAK 2 (Exon 12)						5		G		3		G	0	8	G		home brew (4)	Ipsogen (1), home brew(1)	home brew (3)	home brew (1)
MPL W 515						6		G		2		I	0	8	G		home brew (5), Invader(1)		home brew (2)	
MPL S 505						4		G		2		I	0	6	G		home brew (4)		home brew (2)	
FLT 3 ITD						10		G					0	10	G		home brew (8), IVS(1), Seegene(1)			
FLT 3 D835						9		G					0	9	G		home brew (7), IVS(1), Seegene(1)			
NPM1 mutation						12		G					0	12	G		home brew (12)			
P53					1	1		I					1	1	I		home brew(2)			
IGHV mutation						4N		N		4N		N	0	4N	N		home brew(2) IVS (1)	home brew(1)	home brew (3) IVS (1)	
c-kit						8		G					0	8	G		home brew(8)			
Other ‡						1		I						1	I		home brew(1)			

N*: No clonal band detected

Cons #: R or G based on ≥75% consensus; I if <75% consensus or <3 results

Other ‡ : See critique for details.

Table 2: New York State Molecular Oncology Proficiency Test

Sample: NYS# L/L2 Consensus Summary 3-11.xls

Interpretation: B-cell clone with IGH and IGK gene rearrangements, IGHV hypermutation; EBV positive

Assay	SB				PCR				RT-PCR				All methods			Method used				
	R	G	ind	Cons#	R	G	ind	Cons#	R	G	ind	Cons#	R	G	Cons#	SB	PCR (qualitative)	PCR (quantitative)	RT-PCR (qualitative)	RT-PCR (quantitative)
IGH	1			I	28			R					29	0	R	home brew (1)	Biomed (12), home brew (11), IVS (5)			
IGK					9	1		R					9	1	R		Biomed (9), home brew (1)			
IGL													0	0						
TRB		1		I	4	7		I					4	8	I	home brew (1)	Biomed (9), home brew (2),			
TRG					5	22		G					5	22	G		Biomed (11), home brew (13), IVS (3),			
TRD						1		I					0	1	I					
IGH/BCL2 MBR						13		G					0	13	G		Biomed (3), IVS (2), home brew (7	home brew (1)		
mcr						9	1	G					0	9	G		Biomed (3), IVS (1), home brew (4	home brew (1)		
MBR 3'						3		G					0	3	G		Biomed (3)			
MBR/mcr													0	0						
IGH/CCND1 (Bcl-1)						5		G					0	5	G		home brew (3), IVS (1)	home brew (1)		
BCR/ABL1 p210									1	23		G	1	23	G				home brew (6)	home brew (17), Ipsogen(4), Ceph(1),Asu(1)
p190										21		G	0	21	G				home brew (8)	home brew (12), Ipsogen(4)
p210/190										10		G	0	10	G				home brew (2)	home bre (3), Roche(4),Asu(1) Ipsogen(1)
Abl kinase domain mutation										2		I	0	2	I				home brew (2)	
PML/RARA Long										13		G	0	13	G				home brew (6)	home brew (6), Ipsogen(1)
Short										12		G	0	12	G				home brew (5)	home brew (6), Ipsogen(1)
Variable										4		G	0	4	G				home brew (3)	Ipsogen(1)
Long/Short/Variable													0	0						
MYC t(8;14)													0	0						
AML1/ETO t(8;21)										5		G	0	5	G				home brew (3)	home brew (2)
NPM/ALK t(2;5)													0	0						
IGH/BCL-6													0	0						
ETV6/RUNX1 (Tel-AML1)										2		I	0	2	I				home brew (1)	home brew (1)
EBV					3			R					3	0	R		home brew (2)	Roche (1)		
KSHV/HHV8						3		G					0	3	G		home brew (3)			
HTLV1						2		I					0	2	I		home brew (2)			
CBFB INV(16)/MYH11										2		I	0	2	I				home brew (1)	home brew (1)
E2A-PBX t(1;19) (4;11)										1		I	0	1	I				home brew (1)	
MLL(11q23)/ AF4 (4;11)										2		I	0	2	I				home brew (2)	
JAK 2 (V617F)						24		G		2		I	0	26	G		home brew (17), Ipsogen(3)	home brew(1) Invader(1) Ipsogen(3)	home brew (2)	home brew (1)
JAK 2 (Exon 12)						5		G		3		G	0	8	G		home brew (4)	Ipsogen (1), home brew(1)	home brew (3)	home brew (1)
MPL W 515						6		G		2		I	0	8	G		home brew (5), Invader(1)		home brew (2)	
MPL S 505						4		G		2		I	0	6	G		home brew (4)		home brew (2)	
FLT 3 ITD						10		G					0	10	G		home brew (8), IVS(1), Seegene(1)			
FLT 3 D835						9		G					0	9	G		home brew (7), IVS(1), Seegene(1)			
NPM1 mutation						12		G					0	12	G		home brew (12)			
P53					2			I					2	0	I		home brew(2)			
IGHV mutation					3	1N		R	5			R	8	1N	R		home brew(2) IVS (1)	home brew(1)	home brew (4) IVS (1)	
c-kit						8		G					0	8	G		home brew(8)			
Other ‡						1		I						1	I		home brew(1)			

N*: No clonal band detected

Cons #: R or G based on ≥75% consensus; I if <75% consensus or <3 results

Other ‡ : See critique for details.

Table 3: New York State Molecular Oncology Proficiency Test

Sample: NYS# L/L3 Consensus Summary 3-11.xls

Interpretation: Myeloid with p210 bcr/abl fusion product

Assay	SB				PCR				RT-PCR				All methods			Method used				
	R	G	ind	Cons#	R	G	ind	Cons#	R	G	ind	Cons#	R	G	Cons#	SB	PCR (qualitative)	PCR (quantitative)	RT-PCR (qualitative)	RT-PCR (quantitative)
IGH		1		I		27		G					0	28	G	home brew (1)	Biomed (11), home brew (11), IVS (5)			
IGK					1	9		G					1	9	G		Biomed (9), home brew (1)			
IGL													0	0						
TRB		1		I		10		G					0	11	G	home brew (1)	Biomed (9), home brew (2),			
TRG						26		G					0	26	G		Biomed (10), home brew (13), IVS (3),			
TRD													0	0						
IGH/BCL2 MBR						13		G					0	13	G		Biomed (3), IVS (2), home brew (7	home brew (1)		
mcr						10		G					0	10	G		Biomed (3), IVS (1), home brew (5	home brew (1)		
MBR 3'						3		G					0	3	G		Biomed (3)			
MBR/mcr													0	0						
IGH/CCND1 (Bcl-1)						5		G					0	5	G		home brew (3), IVS (1)	home brew (1)		
BCR/ABL1 p210					1			I	25			R	26	0	R		home brew (1)		home brew (6)	home brew (16), Ipsogen(4), Ceph(1),Asu(1)
p190					1			I	12	9		I	13	9	I		home brew (1)		home brew (7)	home brew (12), Ipsogen(4), Asu(1)
p210/190									7			R	7	0	R				home brew (2)	home bre (2), Roche(4)
Abl kinase domain mutation									2	4		I	2	4	I				home brew (5)	home brew (1)
PML/RARA Long										12		G	0	12	G				home brew (5)	home brew (6), Ipsogen(1)
Short										11		G	0	11	G				home brew (4)	home brew (6), Ipsogen(1)
Variable										3		G	0	3	G				home brew (2)	Ipsogen(1)
Long/Short/Variable													0	0						
MYC t(8;14)													0	0						
AML1/ETO t(8;21)										5		G	0	5	G				home brew (3)	home brew (2)
NPM/ALK t(2;5)													0	0						
IGH/BCL-6													0	0						
ETV6/RUNX1 (Tel-AML1)										2		I	0	2	I				home brew (1)	home brew (1)
EBV						3		G					0	3	G		home brew (2)	Roche (1)		
KSHV/HHV8						3		G					0	3	G		home brew (3)			
HTLV1						2		I					0	2	I		home brew (2)			
CBFB INV(16)/MYH11										2		I	0	2	I				home brew (1)	home brew (1)
E2A-PBX t(1;19) (4;11)										1		I	0	1	I				home brew (1)	
MLL(11q23)/ AF4 (4;11)										1		I	0	1	I				home brew (1)	
JAK 2 (V617F)						23		G		2		I	0	25	G		home brew (16), Ipsogen(3)	home brew(1) Invader(1) Ipsogen(3)	home brew (2)	home brew (1)
JAK 2 (Exon 12)						5		G		3		G	0	8	G		home brew (4)	Ipsogen (1), home brew(1)	home brew (3)	home brew (1)
MPL W 515						6		G		2		I	0	8	G		home brew (5), Invader(1)		home brew (2)	
MPL S 505						4		G		2		I	0	6	G		home brew (4)		home brew (2)	
FLT 3 ITD						9		G					0	9	G		home brew (7), IVS(1), Seegene(1)			
FLT 3 D835						8		G					0	8	G		home brew (6), IVS(1), Seegene(1)			
NPM1 mutation						12		G					0	12	G		home brew (12)			
P53					2			I					2	0	I		home brew(2)			
IGHV mutation						3N		N		4N		N	0	7N	N		home brew(1) IVS (1)	home brew(1)	home brew (3) IVS (1)	
c-kit						8		G					0	8	G		home brew(8)			
Other ‡						1		I						1	I		home brew(1)			

N*: No clonal band detected

Cons #: R or G based on ≥75% consensus; I if <75% consensus or <3 results

Other ‡ : See critique for details.

Table 4: Summary for IGH primer mix

Reagent Source	Mix	L/L1		CONSENSUS	L/L2		CONSENSUS	L/L3		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A		11	G	12		R		10	G
	B		11	G	12		R		10	G
	C		11	G	12		R		10	G
	D		3	G	3		R		2	I
	E		4	G		4	G		2	I
IVS	FR 1		4	G	4		R		3	G
	FR 2		6	G	6		R		5	G
	FR 3		6	G	6		R		5	G
Lab developed	FR 1		2	I	2		I		2	I
(home brew)	FR 2		8	G	8		R		8	G
	FR 3		11	G	11		R		11	G

Table 5: Summary for IGK primer mix

Reagent Source	Mix	L/L1		CONSENSUS	L/L2		CONSENSUS	L/L3		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A	1	8	G	8	1	R		9	G
	B		9	G	8	1	R	1	8	G
Lab developed	A		1	I	1		I		1	I
(home brew)	B		1	I	1		I		1	I

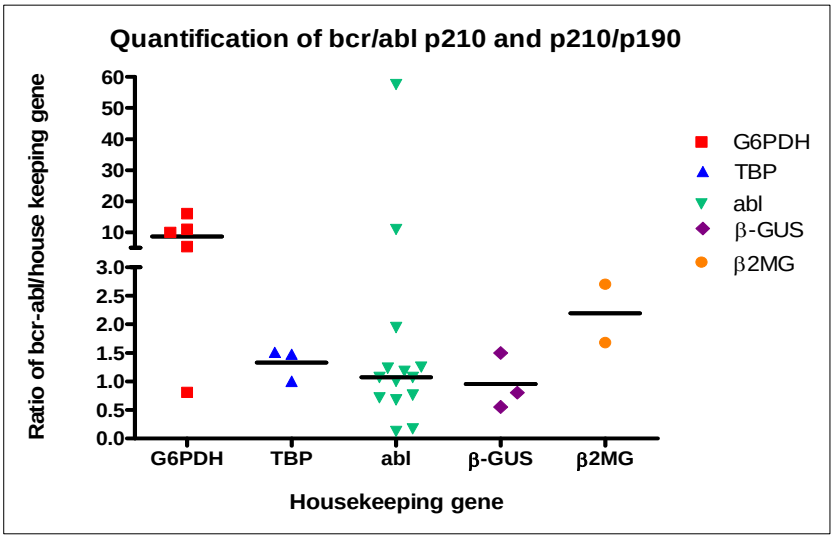
Table 6: Summary for TRG primer mix

Primer Source	Mix	L/L1		CONSENSUS	L/L2		CONSENSUS	L/L3		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A	11		R	2	9	G		10	G
	B	2	9	G		11	G		10	G
IVS	Mix 1	3	1	R	2	1	I		3	G
	Mix 2	3	1	R	2	1	I		3	G
Lab developed	Vγ1-8	5		R		5	G		5	G
(home brew)	Vγ9	1	3	G		4	G		4	G
	Vγ10	3	1	R		4	G		4	G
	Vγ11	1	3	G		4	G		4	G
	Mix 1	3	1	R		4	G		4	G
	Mix 2	3	1	R	1	3			4	G
	Mixed	1				1			1	
	NI	2				2			2	

Table 7: Summary for TRB primer mix

Primer Source	Mix	L/L1		CONSENSUS	L/L2		CONSENSUS	L/L3		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A	9		R	1	8	G		8	G
	B	1	7	G		9	G		8	G
	C	2	6	G	6	2	R		7	G
Lab developed	A	1		I		1	I		1	I
(home brew)	B		1	I		1	I		1	I
	NI		1	I		1	I		1	I

NI: not indicated



NYS PT L/L 3, MCTM 3-11

Results of bcr/abl RT-PCR and QRT-PCR assays (as reported by the labs):

bcr-abl/G6PDH		bcr-abl/TBP		bcr-abl/BCR		bcr-abl/abl		bcr-abl/B-GUS		bcr/abl/B2MG		Not Indicated	
p210	p190	p210	p190	p210	p190	p210	p190	p210	p190	p210	p190	p210	p190
81%		147.75%	3.260%	R		1.232	0.0002	0.554	0.0016	168.19%	0.019%	72.00%	
5.45		151%				100%	0.03%	0.803	R	270.41%	0.096%	85.00%	
9.99		100%				76.09%	0.04%	1.5	R			R	
1600%						67.59%	0.02%						
11%						107%	0.02%						
						16.67%	0.01%						
						1.945							
						118.05%							
						10.937							
						12.67%							
						71.42%							
						125.00%							
						107.00%							
						R	R						
						57.562							

Figure 2 and table: PT L/L 3 bcr/abl quantification. For the figure, all % numbers were converted to a ratio by dividing by 100.

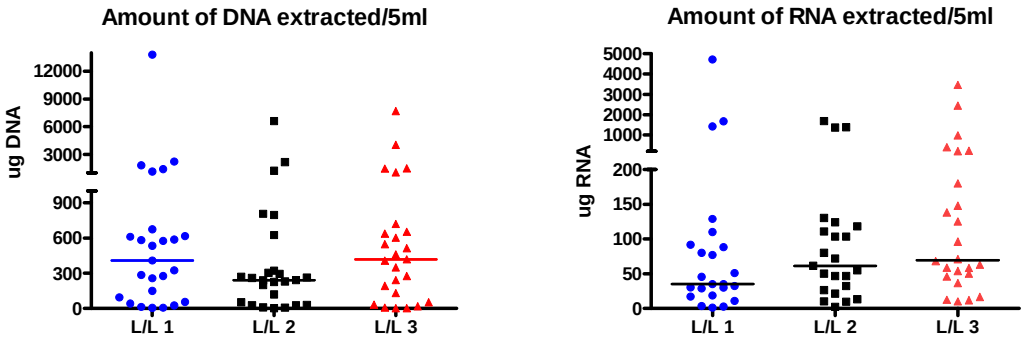


Figure 1. NYS MCTM PT 11-10 DNA and RNA yields. The yields were converted to ug DNA and RNA per 5 ml blood.

	LL1	LL2	LL3		LL1	LL2	LL3
	DNA	DNA	DNA		RNA	RNA	RNA
Median	408.0	241.0	418.8		35.1	61.3	69.4
Max	13820.0	6600.0	7690.0		4710.0	1690.0	3465.0
Min	5.0	2.5	2.5		1.4	2.1	10.1