NEW YORK
state department of
HEALTH

Nirav R. Shah, M.D., M.P.H.
Commissioner

Sue Kelly
Executive Deputy Commissioner

Molecular and Cellular Tumor Marker Proficiency Test Event
MCTM 3-2012
Summary of results[1]

May 21, 2012

Dear Laboratory Director,

Below is a summary and discussion of the New York State Molecular and Cellular Tumor Markers proficiency test event MCTM 3-2012 from March 20, 2012.

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 38 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. For IGHV only: **H**: clonal band detected and hypermutated; **U**: clonal band detected, but not hypermutated; **N**: no clonal band detected. **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. 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 was 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 separate report cards were generated. The assay score is indicated in the

[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

'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 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 apparently false positives.

NYS#L/L 2012-01 (Table 1):
B-cell tests: For IGH and IGK there was unanimous agreement that these genes were not rearranged. Similarly, no laboratory found an IGH/BCL-2 and/or IGH/CCND1 translocation. Thus, the overall consensus was that this sample did not contain cells with any immunoglobulin gene rearrangements.

T-cell tests: all 24 laboratories that tested for TRG by PCR found a rearrangement, possibly in the Vγ1-8 region targeted by the Biomed-2 tube A and home brew Vγ1-8 primers (Table 5). Eleven out of twelve labs (92%) that tested for TRB reported a rearrangement by PCR that was detected by one or more of the three Biomed-2 tubes A, B, and C although there seemed to be no clear consensus of which primers were positive or negative (Table 4); one lab reported an indeterminate result. These results suggest that this sample contained cells with T-cell receptor gamma and beta gene rearrangements.

Various mutations (Table 6): Eight labs reported the p.G12D, c.35G>A, mutation in KRAS. One lab reported two mutations in RUNX1, c.236T>C, p.V79A and c.1108G>A, p.A370T, and one of two labs reported three mutations in TP53, p.R175H, p.R248Q, and p.R282W, whereas the second lab did report TP53 as wild type.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal T-cell population with a KRAS mutation, and possibly TP53 and RUNX1 mutations. This conclusion is in agreement with the result from Flow Cytometry, which indicated a precursor T-lymphoblastic leukemia that expressed surface antigens CD4, CD5, CD7, CD38 and CD45.

NYS#L/L 2012-02 (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 A to C that target all three framework regions, but not with the tube E that targets only the DH 7 region, whereas results

with tube D primers were indeterminate. Similarly, labs that used the IVS (not Biomed-2) or LDT primers detected a rearrangement in all three framework regions (Table 2). Rearrangements in IGK were only detected with the Biomed-2 tube A primers, but not the tube B primers (Table 3). Four labs reported an IGH/CCND1 translocation, whereas no lab reported a translocation involving the IGH/BCL2 locus. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements, and the IGH/CCND1 (bcl-1) translocation.

IGHV: Eight out of nine labs (89%) reported IGHV hypermutation, and assigned it to the IGHV4-59 family with a mutation rate ranging from 6.1-10.2%.

T-cell tests: 23/24 (96%) and 12/12 (100%) laboratories that tested for TRG or TRB, respectively, by PCR found no rearrangement. These results suggest that this sample did not contain cells with TRB and/or TRG gene rearrangements.

EBV: All four labs that tested for detected the presence of EBV sequences by PCR.

Various mutations (Table 6): Two labs detected the TP53 exon 7 p.M237I (c.14038G>T) mutation by sequencing. Please note, according to the IARC TP53 database (http://www-p53.iarc.fr/MutationValidation.asp?Mutant=M237I) this mutation also corresponds to genomic mutation g.13348G>T. No other mutations were detected.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal B-cell population with hypermutation in the IGHV region, IGH/CCND1 translocation, and the presence of EBV DNA, consistent with mantle cell lymphoma. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of a mature B-cell clone positive for CD19, CD20, CD22, CD23, CD38, CD45, and HLA-DR with kappa expression.

NYS#L/L 2012-03 (Table 1):
B-cell tests: 17 out of 26 laboratories (65%) reported an IGH rearrangement by PCR; five labs reported no rearrangement and four reported indeterminate results. However, there was no consistent pattern based on primers and/or kits used with the possible exception of the Biomed-2 tube D (Table 2). All nine labs that tested for IGK by PCR found a rearrangement, possibly in the Vk, Jk, and Kde regions detected by both Biomed-2 A and B tubes (Table 3). No lab reported a translocation involving the IGH/BCL2 or IGH/CCND1 loci. Thus, the consensus was that this sample contained cells with an immunoglobulin kappa gene rearrangement and possibly an IGH rearrangement, but those results were below the 80% concordance required for a consensus.

T-cell tests: 17 out of 24 laboratories (71%) that tested for TRG by PCR found a rearrangement with the Biomed-2 tube A primers (Table 5). In contrast, all twelve labs that tested for TRB reported no rearrangement. These results suggest that this sample may have contained cells with a TRG gene rearrangement, though it did not attain the required 80% concordance for a consensus.

Various mutations (Table 6): Two labs reported a mutation in P53, one calling it 810_811insT (p.E271fs*), the other p.F270fs (c.14479insT), leading to a frame shift at codons 270/271. It is unclear whether these represent the same mutations. Interestingly, the only similar mutation described in either the Cosmic or IARC TP53 database is c.810_811insN, p.E271fs*35 (http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=mut_summary&id=45666). In addition, two labs reported a mutation in NRAS, in codon 12, namely p.G12C (c.34G>T).

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal population of possibly immature or early B-cell lineage. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of an immature or early B-cell clone positive for CD10, CD19, CD20, CD22, CD38 and HLA-DR with no surface expression of kappa, lambda or CD5.

The attached tables show summaries of the results in aggregate (Table 1) as well as for each individual primer mix for the B- and T-cell tests (Tables 2-5). Furthermore, Table 6 shows a summary of the mutation results, and Tables 7 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. DNA yields from samples L/L1, 2, and 3 ranged from a minimum of 0.2, 0.42, and 0.32 µg/ml to a maximum of 854, 723, and 438 µg/ml, respectively, corresponding to a 1369- to 4270-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 0.5, 0.75, and 0.6 µg/ml to 404, 380, and 265 µg/ml, respectively, corresponding to a 442- to 808-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, that you indicate the volume correctly, 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. 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. A few labs did not indicate the methods and/or reagents that they used for their assays or failed to give the overall result in the first column. 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 (not Biomed-2) (identified as gene rearrangement assays in their catalog) or IVS (Biomed-2) (identified as gene clonality assays in their catalog); for the purpose of this PT evaluation they are not considered lab developed even if you obtain the individual primer tubes separately as ASR reagents instead of as part of a RUO kit. This will make it easier to compare the performance of individual primer mixes. Finally, we ask that you

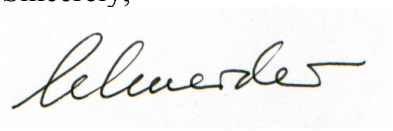
analyze the samples by all molecular tests performed in your lab for which you hold or have applied for a NYS permit.

If you have any questions, comments or suggestions, you may contact me by phone or email at 518-474-2088 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.

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	**Due Date**
October 23, 2012	November 21, 2012

Sincerely,



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New York State Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 3-2012
Table 1: Summary of results

Assay / Sample	L/L 2012-01					L/L 2012-02					L/L 2012-03				
	R/H	G/U	I	N	Cons 01#	R/H	G/U	I	N	Cons 02#	R/H	G/U	I	N	Cons 03#
IGH		26			G	26				R	17	5	4		I
IGK		9			G	9				R	9				R
TRB	11		1		R		12			G		12			G
TRG	24				R	1	23			G	17	7			I
IGHV				8	N	8			1	H	1	1	2	5	I
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
IGH/BCL2		10			NEG		9	1		NEG		10			NEG
IGH/CCND1		4			NEG	4				POS		4			NEG
IGH/MYC															
	MUT	WT	I			MUT	WT	I			MUT	WT	I		
JAK2 V617F	1	31			WT	1	31			WT	1	31			WT
JAK2 Exon 12		9			WT		9			WT		9			WT
MPL W515		13			WT		13			WT		13			WT
MPL S505		9			WT		9			WT		9			WT
FLT3 ITD		6			WT		6			WT		6			WT
FLT3 D835		5			WT		5			WT		5			WT
NPM1		11			WT		11			WT		11			WT
CEBPA		6			WT		6			WT	1	5			WT
IDH1		4			WT		4			WT		4			WT
KIT		7			WT	1	6			WT		7			WT
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
BCR/ABL1		30			NEG	1	29			NEG		30			NEG
BCR/ABL1 p210		20			NEG		20			NEG		20			NEG
BCR/ABL1 p190		19			NEG	1	18			NEG		19			NEG
BCR/ABL1 p210/p190		4			NEG		4			NEG		4			NEG
	MUT	WT	I			MUT	WT	I			MUT	WT	I		
ABL Kinase domain		2	1		I		2	1		I		2	1		I
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
PML/RARA		13			NEG		13			NEG		13			NEG
PML/RARA Long		10			NEG		10			NEG		9			NEG
PML/RARA short		10			NEG		10			NEG		9			NEG
PML/RARA variable		4			NEG		4			NEG		4			NEG
PML/RARA L/S/V															
AML1/ETO		5			NEG		5			NEG		5			NEG
NPM1/ALK															
ETV6/RUNX1		1			I		1			I		1			I
CBFB/MYH11		3			NEG		3			NEG		3			NEG
TCF3/PBX1		1			I		1			I		1			I
MLL/AF4		2			I		2			I	1	1			I
	MUT	WT	I			MUT	WT	I			MUT	WT	I		
TP53	1	1			I	2				I	2				I
KRAS	8				MUT		7			WT		7			WT
NRAS		2			I		2			I	2				I
HRAS		2			I		2			I		2			I
BRAF		6			WT		6			WT		6			WT
	POS	NEG	I			POS	NEG	I			POS	NEG	I		
EBV		4			NEG	4				POS		4			NEG
Interpretation:	Clonal T-cell population with TRB and TRG rearrangements and KRAS mutation					Clonal B-cell population with IGH and IGK rearrangements, IGH/CCND1 translocation, and presence of EBV. Consistent with Mantle Cell Lymphoma.					Clonal B-cell population with IGK rearrangement				
Comments															

R: rearranged/clonal band detected; G: germline/no clonal band detected; WT: wild-type; MUT: mutated; NEG: neagtive or not detected; POS: positive or detected; I: indeterminate, a clear interpretation is not possible.
For IGHV only: H: clonal band detected and hypermutated; U: clonal band detected, but not hypermutated; N: no clonal band detected.
Cons#: Consensus based on ≥80% concordance; I if no consensus or <3 results

Table 2. Summary for IGH primer mixes

	L/L 2012-01			L/L 2012-02			L/L 2012-03		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT FR 1	0	1	**I**	1	0	**I**	1	0	**I**
LDT FR 2	0	7	**G**	7	0	**R**	2	4	**I**
LDT FR 3	0	10	**G**	10	0	**R**	4	6	**I**
Biomed-2 Tube A	0	8	**G**	8	0	**R**	4	2	**I**
Biomed-2 Tube B	0	9	**G**	9	0	**R**	4	2	**I**
Biomed-2 Tube C	0	8	**G**	8	0	**R**	3	3	**I**
Biomed-2 Tube D	0	3	**G**	1	2	**I**	3	0	**R**
Biomed-2 Tube E	0	4	**G**	0	4	**G**	0	4	**G**
IVS FR 1	0	7	**G**	7	0	**R**	5	2	**I**
IVS FR 2	0	8	**G**	9	0	**R**	4	1	**R**
IVS FR 3	0	9	**G**	8	1	**R**	5	2	**I**

Table 3. Summary for IGK primer mixes

	L/L 2012-01			L/L 2012-02			L/L 2012-03		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT Tube A	0	1	**I**	1	0	**I**	1	0	**I**
LDT Tube B	0	1	**I**	0	1	**I**	1	0	**I**
Biomed-2 Tube A	0	8	**G**	8	0	**R**	8	0	**R**
Biomed-2 Tube B	0	8	**G**	1	7	**G**	8	0	**R**

Table 4. Summary for TRB primer mixes

	L/L 2012-01			L/L 2012-02			L/L 2012-03		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT Tube A	2	0	**I**	0	2	**I**	0	2	**I**
LDT Tube B	0	1	**I**	0	1	**I**	0	1	**I**
Biomed-2 Tube A	6	3	**I**	0	9	**G**	0	9	**G**
Biomed-2 Tube B	4	4	**I**	0	9	**G**	0	9	**G**
Biomed-2 Tube C	6	1	**R**	0	8	**G**	0	8	**G**

Table 5. Summary for TRG primer mixes

	L/L 2012-01			L/L 2012-02			L/L 2012-03		
	R	**G**	**cons**	**R**	**G**	**cons**	**R**	**G**	**cons**
LDT Vy1-8	7	0	**R**	0	7	**G**	3	4	**I**
LDT Vy9	1	4	**G**	1	4	**G**	1	4	**G**
LDT Vy10	2	3	**I**	0	5	**G**	1	4	**G**
LDT Vy11	2	1	**I**	0	4	**G**	1	3	**I**
Biomed-2 Tube A	12	0	**R**	0	12	**G**	10	2	**R**
Biomed-2 Tube B	1	11	**G**	0	12	**G**	1	11	**G**
IVS Mix 1	1	0	**I**	0	1	**I**	1	0	**I**
IVS Mix 2	0	1	**I**	0	1	**I**	0	1	**I**

Table 6: Summary of other mutation assay results

Gene	LL1	# of labs		LL2	# of labs		LL3	# of labs
TP53	p.R175H; p.R248Q; R282W WT	1 1		c.14038G>T, p.M237I	2		810_811insT, p.E271fs* c.144479insT, p.270fs	1 1
KRAS	c.35G>A, p.G12D	8						
NRAS							c.34G>T, p.G12C	2
HRAS								
BRAF								
PIK3CA								
EGFR								
WT1								
PDGFRA								
JAK2 ex13								
RUNX1	c.236T>C, pV79A; c.1108G>A, p.A370T	1						
IDH2								

NOTE: each gene listed was analyzed by at least one laboratory for the presence of mutations; no entry means no mutation was reported.

Table 7: Summary of methods and reagents used

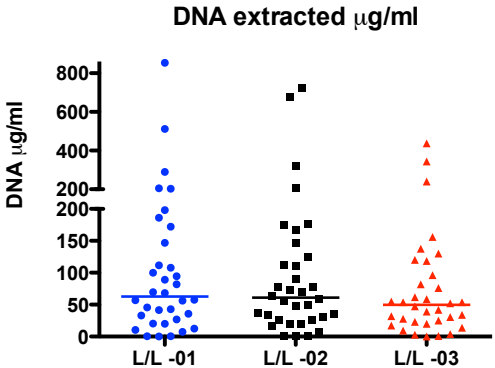
	Total	SB	PCR	Seq	PCR Seq	Lab developed	IVS (Biomed-2)	IVS (not Biomed-2)	Lab developed and IVS (Biomed-2)	Qualitative	Quantitative
IGH	29	2	27	0	0	10	9	8	1		
IGK	9	0	9	0	0	1	8	0	0		
TRB	13	2	11	0	0	3	9	0	0		
TRG	25	0	25	0	0	13	11	0	0		
IGHV	9	0	2	5	2	6	3	0	0		
IGH/BCL2	11	0	11	0	0	7	4	0	0		
IGH/CCND1	4	0	4	0	0	4	0	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	Ipsogen (Qiagen)	Seegene	Qualitative	Quantitative
JAK2 V617F	33	26	2	3	0	1	25	8	0	19	14
JAK2 Exon 12	9	4	1	2	0	1	9	0	0	0	0
MPL	13	6	0	4	1	2	13	0	0		
FLT3 ITD	6	6	0	0	0	0	6	0	0	0	0
FLT3 D835	5	5	0	0	0	0	5	0	0	0	0
NPM1	11	11	0	0	0	0	11	0	0	1	0
CEBPA	6	3	0	2	1	0	6	0	0	2	0
IDH1	4	1	0	3	0	0	4	0	0	0	0
KIT	0	0	0	0	0	0	0	0	0		

	Total	PCR	RT-PCR	Seq	PCR Seq	RT-PCR Seq	Lab developed	Ipsogen (Qiagen)	Roche	Cepheid	Asuragen	Qualitative	Quantitative	IS Normalized
BCR/ABL1	31	0	30	0	0	0	22	6	3	1	1	3	28	7
ABL Kinase domain	4	0	1	1	0	2	4	0	0	0	0	1	0	0
PML/RARA	13	0	13	0	0	0	12	1	0	0	0	4	9	0
AML1/ETO	5	0	5	0	0	0	5	0	0	0	0	3	2	0
NPM1/ALK	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ETV6/RUNX1	1	0	1	0	0	0	1	0	0	0	0	0	1	0
CBFB/MYH11	3	0	3	0	0	0	3	0	0	0	0	2	1	0
TCF3/PBX1	1	0	1	0	0	0	1	0	0	0	0	1	0	0
MLL/AF4	2	0	2	0	0	0	2	0	0	0	0	2	0	0

	Total	PCR	Seq	PCR Seq	Lab developed	Other
TP53	2	0	1	1	0	2
KRAS	8	4	1	3	4	4
NRAS	2	0	0	2	0	2
HRAS	2	1	0	1	0	2
BRAF	7	4	1	2	1	6
EBV	4	4	0	0	1	3

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



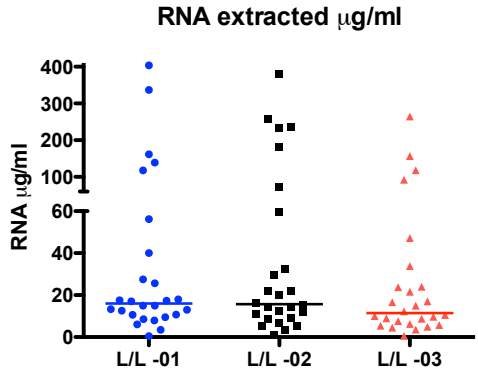


Figure 1. NYS MCTM PT 3-12 DNA and RNA yields. The yields were converted to ug DNA and RNA per 1 ml blood.

	L/L 2012-01	L/L 2012-02	L/L 2012-03		L/L 2012-01	L/L 2012-02	L/L 2012-03
	DNA	DNA	DNA		RNA	RNA	RNA
Median	62.9	61.2	49.9	Median	16.0	15.7	11.4
Min	0.20	0.42	0.32	Min	0.50	0.75	0.60
Max	854.0	722.5	438.0	Max	404.0	380.0	264.8