



# STATE OF NEW YORK DEPARTMENT OF HEALTH

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*Executive Deputy Commissioner*

## **Molecular and Cellular Tumor Marker Proficiency Test Program MCTM 10-08 Summary of results<sup>1</sup>**

December 22, 2008

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from October 28, 2008, MCTM 10-08.

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 33 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) or **R** (Rearranged/positive) 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 3). **I** (Indeterminate) is shown if no consensus was reached because only one lab performed a test, or if the difference between the number of labs reporting R or G is not sufficient to derive a clear consensus. 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 as 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 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, and the numerator is the number of samples for which you agree 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

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<sup>1</sup> 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

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' 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.

#### **NYS#L/L 1 (Table 1):**

B-cell tests: For IGH, 24 out of 25 (96%) laboratories reported no rearrangement by PCR. The one lab that reported a rearrangement by PCR used the Biomed-2 tube E (Table 4); however, the two other labs that also assayed the tube E did not find a rearrangement. Although two out of three (67%) labs found a rearrangement by SB (1=Dako, 1=HB), there was no consensus since the third lab (by Dako) did not find a rearrangement. The two labs that used FISH also reported no rearrangement. All six labs that tested for IgKappa (IGK) by PCR found no rearrangement, as did the one laboratory that tested for IGK by SB. The one lab that also tested for IgLambda (IGL) by SB found no rearrangement. All laboratories that tested for IGH/BCL2 by various methods reported no translocation at any of the three breakpoint clusters. Similarly, none of the eight laboratories that tested for the IGH/CCND1 translocation (also known as Bcl-1) by any method found a rearrangement. Thus, the overall consensus was that this sample did not contain an immunoglobulin gene rearrangement.

T-cell tests: 21 out of 25 (84%) laboratories that tested for TcRGamma (TRG) by PCR found a rearrangement, possibly in the V $\gamma$ 9 and 10 regions that were detected by all labs that used the Biomed-2 tubes A and B or home brew V $\gamma$  9 and 10 primers (Table 6). Of the four labs that did not find a TRG rearrangement, three did not indicate what primers they used, and one used the IVS mix 1. Four out of six (67%) labs that tested for TcRBeta (TRB) reported a rearrangement by PCR, possibly in the D $\beta$  and J $\beta$ 1 region detected by the Biomed-2 tubes A and C (Table 7). However, only two out of the four (50%) labs that used SB (1=Dako, 1=HB) detected a TRB rearrangement, whereas the other two reported no rearrangement (1=Dako, 1=not indicated). Therefore, there was no clear consensus from both methods whether this sample exhibited a TRB rearrangement or not. Furthermore, there is no evidence from Flow Cytometry of a productive TcR beta gene expression. The one laboratory that tested for TcRDelta (TRD) by SB found no rearrangement. These results suggest that this sample contained cells with T-cell receptor gamma and possibly beta gene rearrangements.

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 a poorly differentiated aberrant cell type that expresses T cell markers (cCD3, CD5 and CD7) and myeloid/monocytic markers (CD7 and CD15).

**NYS#L/L 2 (Table 2):**

B-cell tests: For IGH, there was unanimous agreement that this locus was rearranged (25=PCR, 2=SB, 2=FISH). Rearrangements were detected by PCR using the Biomed-2 tubes A to D that target all three framework regions and also six DH regions, except the tube E that targets only the DH 7 region. Similar results were obtained by the labs that used either the original IVS or home brew primers (Table 4). Similarly, all seven labs that tested for IGK (PCR=6, SB=1) reported a rearrangement by both Biomed-2 tubes A and B (Table 5) or SB, as did the one lab that also tested for IGL by SB. With one exception, no translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any methods. Thus, these results suggest that this sample contained a B-cell clone with IGH, IGK, and possibly IGL gene rearrangements.

T-cell tests: All 25 laboratories that tested for TcRGamma (TRG) by PCR found no rearrangement. Likewise, all nine labs that tested for TcRBeta (TRB) reported no rearrangement (3=SB, 6=PCR), though one lab detected a possible rearrangement with the Biomed-2 tube A, but concluded that this was not significant as the intensity of the band was below that of their sensitivity control of 10% (Table 7). The one laboratory that tested for TcRDelta (TRD) by SB also found no rearrangement. These results suggest that this sample did not contain cells with T-cell receptor gene rearrangements.

MYC t(8;14): All three labs that used FISH identified the presence of the translocation, which is a marker for Burkitt lymphoma.

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

P53: Two labs detected the Y163C mutation in the P53 gene by PCR and sequencing; in contrast the two labs that used FISH reported no alteration of the P53 locus on chromosome 17.

IGHV mutation: All eight labs that tested reported IGHV hypermutation (4=PCR, 4=RT-PCR), of which seven assigned it to the IGHV3-11 family, and three reported mutation rates between 8.0-9.2%. The rest did not specify the family or the mutation rate.

One lab that used FISH reported 18% and 88.7% of cells with signals for CFBF INV(16)/MYH11 and MLL(11q23)/AF4(4;11), respectively.

The results from all other tests performed were negative.

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

**NYS#L/L 3 (Table 3):**

B-cell tests: For IGH, all 25 laboratories that used PCR reported a rearrangement, which was confirmed by the two laboratories that used SB. Rearrangements were detected with the Biomed-2 tubes A, B, and C, but not D and E, and also the IVS and home brew primers that target all three framework regions (Table 4). Interestingly, the two labs that used FISH reported no rearrangement, however, they also used SB and PCR, respectively, and reported a rearrangement with those methods. Similarly, all seven labs that

tested for IGK (PCR=6, SB=1) reported a rearrangement with both Biomed-2 tubes A and B primers (Table 5) or SB, whereas the one lab that also tested for IGL by SB found no rearrangement. No translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any methods. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: All 25 laboratories that tested for TRG by PCR found no rearrangement. Four out of six (67%) labs that tested for TRB by PCR also indicated no rearrangement; however, two out of these four labs found a positive band with the Biomed-tube C, but reported G since they concluded that the tube C detects an incomplete (D-J) TCR beta gene rearrangement that usually has a poor correlation with the neoplastic process in the T lineage. Two out of three labs detected a rearrangement by SB. Thus, while a majority of labs detected a possible TRB gene rearrangement analytically its clinical significance is unclear. Furthermore, there is no evidence from Flow Cytometry of a productive TcR beta gene expression. The one lab that tested for TRD found no rearrangement by SB. These results suggest that this sample did not contain cells with T-cell receptor gene rearrangement.

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

IGHV mutation: Seven out of eight (88%) labs reported IGHV hypermutation. Of those, six labs assigned the mutations to the IGHV1-46 family, and three reported mutation rates between 8.65 to 10.68%. The rest did not specify the family or the mutation rate. The one lab that failed to detect a clonal band used home brew primers, suggesting a problem with their PCR.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a B-cell clone with IGHV hypermutation and presence of EBV virus sequence. The overall diagnosis is consistent with the results from Flow Cytometry, which indicated an early B cell.

In **summary**, there was good agreement between the molecular and the flow cytometry results. However, the analysis by molecular methods clearly provided additional information by finding that sample L/L 2 contained EBV sequences, MYC gene translocation and IGHV hypermutation, and that L/L 3 contained EBV sequences and IGHV hypermutation. Conversely, the results from Flow Cytometry help with interpreting the analytic finding in L/L 3 that TRB rearrangement is rearranged but not productively expressed. Thus, these two approaches are complementary rather than redundant. In this PT, we asked for an overall diagnosis for each sample, and required you to provide this information as in the patient test report. In general, there was a good consensus in the diagnoses given. Starting with the next PT in February 2009, for those labs that perform both Flow Cytometry and molecular analysis, we ask that you incorporate the molecular result in the Flow report. While we recognize that PT reporting is somewhat artificial, we still urge you to make sure that your interpretation matches the analytical results. 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 1.5, 5.3, and 9.8 µg per 5 ml specimen to a maximum of 6156, 5580, and 7932 µg, respectively, corresponding to a 4104- to 754-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 8.55, 7, and 7.38 µg to 1623, 1860, and 1781

µg, respectively, corresponding to a 189- to 265-fold difference between lowest and highest yield for each sample. Please make sure that you report the DNA/RNA yields calculated for the entire 5 ml sample even if you only extract it from a smaller aliquot. Presumably, the methods used for DNA and RNA isolation contributed to this wide range. However, 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, some general comments. There still seems to be some confusion as to where to write your results. Please note: RT stands for reverse transcription, 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. Some labs that used FISH only entered their results as a percentage, e.g. 76%; those labs should indicate whether their interpretation of this result is R or G. 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. 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](mailto: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](mailto:smchale@wadsworth.org), or Dr. Rong Yao at (518) 474-1744 or [yaor@wadsworth.org](mailto:yaor@wadsworth.org).

The dates for the Molecular and Cellular Tumor Marker PT mail-out in 2009 are:

**Mail-out date**

February 29  
June 22  
October 26

**Due Date**

March 23  
July 20  
November 23

Sincerely,

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**Table 1: New York State Molecular Oncology Proficiency Test -Summary of Results**

**Sample: NYS# L/L 1 (October 2008)**

Overall diagnosis: T-cell clone with TRG rearrangements																									
Assay	SB				PCR				RT-PCR				FISH				All methods			Method used					
	R	G	ind	Cons	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)	FISH
IGH	2	1	I	I	1	24	G	G				I	2		G	3	27	I/G/G	Dako (2), home brew (1)	Biomed (9), home brew (13), IVS (4)				Vysis (2)	
IGK		1	I	I		6	G	G				I			I	0	7	G	home brew (1)	Biomed (6)					
IGL		1	I	I			I	I				I			I	0	1	I	home brew (1)						
TRB	2	2	I	I	4	2	I	I				I			I	6	4	I	home brew (2), Dako (2)	Biomed (5), home brew (1)					
TRG			I	I	21	4	R	R				I			I	21	4	R		Biomed (5), home brew (17), IVS (2), NI (1)					
TRD		1	I	I			I	I				I			I	0	1	I	home brew (1)	home brew (1)					
IGH/BCL2	MBR	1	I	I		14	G	G				I			G	0	18	G	home brew (1)	Biomed (2), IVS (1), home brew (8)	home brew (2)				
	mcr		I	I		12	G	G				I			I	0	12	G		Biomed (2), IVS (1), home brew (7)	home brew (2)				
	MBR 3'		I	I		2	G	G				I			I	0	2	G		Biomed (2)					
	MBR/mcr		I	I			I	I				I	3		G	0	3	G						Vysis (3)	
IGH/CCND1 (Bcl-1)		1	I	I		6	G	G				I	3		G	0	10	G	home brew (1)	home brew (3), other (1)	home brew (2)			Vysis (2)	
BCR/ABL1	p210	1	I	I			I	I	26			G			I	0	27	G	home brew (1)			home brew (7), IVS (1)	home brew (17), Ipsiogen(3), Caspheid(1), NI (1)		
	p190		I	I			I	I	22			G			I	0	22	G				home brew (8), Ipsiogen (1)	home brew (12), Ipsiogen(1)		
	p210/190		I	I			I	I	7			G	4		G	0	11	G				home brew (1), Roche(1)	home brew (3), Roche(2)	Vysis (4)	
Abl kinase domain mutation			I	I			I	I	2			G			I	0	2	G					NI (2)		
PML/RARA	Long		I	I			I	I	12			G			I	0	12	G				home brew (6), Ipsiogen(1)	home brew (5)		
	Short		I	I			I	I	10			G			I	0	10	G				home brew (5)	home brew (5)		
	Variable		I	I			I	I	4			G			I	0	4	G				home brew (3)	home brew (1)		
	Long/Short/Variable		I	I			I	I	1			I	3		G	0	4	G				home brew (1)		Vysis (3)	
MYC t(8;14)			I	I			I	I				I	3		G	0	3	G						Vysis (3)	
AML1/ETO t(8;21)			I	I			I	I	7			G	3		G	0	10	G				home brew (5)	home brew (1)	Vysis (3)	
NPM/ALK t(2;5)			I	I			I	I	1			I	1		I	0	2	G				home brew (1)		Vysis (1)	
IGH/BCL-6			I	I			I	I				I	2		G	0	2	G						Vysis (2)	
ETV6/RUNX1 (Tel-AML1)			I	I			I	I	3			G	3		G	0	6	G				home brew (2)	home brew (1)	Vysis (3)	
EBV			I	I	4		G	G				I			I	0	4	G	home brew (2)	Roche (1), other (1)					
KSHV/HHV8			I	I	3		G	G				I			I	0	3	G	home brew (3)						
HTLV1			I	I	2		G	G				I			I	0	2	G	home brew (2)						
CBFB INV(16)/MYH11			I	I			I	I	4			G	4		G	0	8	G				home brew (3)	home brew (1)	Vysis (3)	
E2A-PBX t(1;19) (4;11)			I	I			I	I				I			I	0	0	I				home brew (1)			
MLL(11q23)/ AF4 (4;11)			I	I			I	I	3			G	4		G	0	7	G				home brew (3)		Vysis (4)	
JAK 2 (V617F)			I	I	26		G	G	1			I			I	0	27	G	home brew (17), IVS(4), Ipsiogen(2)	home brew(2), Eragen(2), Invader(1)		home brew (1)	home brew (1)		
JAK 2 (Exon 12)			I	I			I	I	1			I			I	0	1	I				home brew (1)	home brew (1)		
MPL W 515			I	I	2		G	G	1			I			I	0	3	G	home brew (1), Invader (1)			home brew (1)			
MPL S 505			I	I			I	I	1			I			I	0	1	I				home brew (1)			
FLT 3 ITD			I	I	14		G	G				I			I	0	14	G	home brew (10), IVS(3), Seegene(1)						
FLT 3 D835			I	I	12		G	G				I			I	0	12	G	home brew (8), IVS(3), Seegene(1)			home brew (9)			
NPM1 mutation			I	I	9		G	G				I			I	0	9	G							
P53			I	I	2		G	G				I	2		G	0	4	G	home brew(2)					Vysis (2)	
IgVH mutation			I	I	3		N*	N*	4			N*			I	0	7	N*	home brew(2), IVS(1)	IVS(1)		home brew (3), IVS (1)			
c-kit			I	I	3		G	G				I			I	0	3	G	home brew (3)						
Other:(MYC,RARA,ALK, Bcl-6), (HFE, RB1, etc)			I	I	1		I	I				I	1		I	0	2	I	home brew (1)					Vysis (1)	

\* No clonal band detected

**Table 2: New York State Molecular Oncology Proficiency Test -Summary of Results**

**Sample: NYS# L/L 2 (October 2008)**

Overall diagnosis: B-cell clone with IGH and IGK rearrangements, EBV positive, MYC and IgVH mutations																									
Assay	SB				PCR				RT-PCR				FISH				All methods			Method used					
	R	G	ind	Cons	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)	FISH
IGH	2			R	25			R				I	2			R	29	0	R	Dako (1), home brew (1)	Biomed (9), home brew (13), IVS (7)				Vysis (2)
IGK	1			I	6			R				I				I	7	0	R	home brew (1)	Biomed (6)				
IGL	1			I				I				I				I	1	0	I		home brew (1)				
TRB		3		G	6			G				I				I	0	9	G	home brew (2), Dako (1)	Biomed (5), home brew (1)				
TRG				I	25			G				I				I	0	25	G		Biomed (5), home brew (17), IVS (2), NI (1)				
TRD	1			I				I				I				I	1	0	I	home brew (1)					
IGH/BCL2	MBR	1		I	1	12		G				I				I	1	13	G	home brew (1)	Biomed (2), IVS (1), home brew (8)	home brew (1)			
	mcr			I		11		G				I				I	0	11	G		home brew (2)				
	MBR 3'			I		2		G				I				I	0	2	G						
	MBR/mcr			I				I				I	3			G	0	3	G						Vysis (3)
IGH/CCND1 (Bcl-1)		1		I	6			G				I	3			G	0	10	G	home brew (1)	home brew (3), other (1)	home brew (2)			Vysis (3)
BCR/ABL1	p210			I				I	25			G				I	0	25	G	home brew (1)			home brew (7), Ipsogen (1)	home brew (16), Ipsogen(1), Caspiel(1), NI (1)	
	p190			I				I	21			G				I	0	21	G				home brew (8), Ipsogen (1)	home brew (11), Ipsogen(1)	
	p210/190			I				I	7			G	3			G	0	10	G				home brew (1), Roche(1)	home brew (3), Roche(2)	Vysis (3)
	Abl kinase domain mutation			I				I	2			G				I	0	2	G				NI (2)		
PML/RARA	Long			I				I	12			G				I	0	12	G				home brew (6), Ipsogen (1)	home brew (5)	
	Short			I				I	10			G				I	0	10	G				home brew (5)	home brew (5)	
	Variable			I				I	4			G				I	0	4	G				home brew (3)	home brew (1)	
Long/Short/Variable			I				I	1			I	3			G	0	4	G				home brew (1)		Vysis (3)	
MYC t(8;14)				I				I		3		I			R	3	0	R							Vysis (3)
AML1/ETO t(8;21)				I				I	7			G	3			G	0	10	G				home brew (5)	home brew (2)	Vysis (3)
NPM/ALK t(2;5)				I				I	1			I	1			I	0	2	G				home brew (1)		Vysis (1)
IGH/BCL-6				I				I				I	2			G	0	2	G						Vysis (2)
ETV6/RUNX1 (Tel-AML1)				I				I	3			G	2			G	0	5	G				home brew (2)	home brew (1)	Vysis (2)
EBV				I	4			R				I				I	4	0	R		home brew (2)	home brew (2)			
KSHV/HHV8				I	3			G				I				I	0	3	G		home brew (3)				
HTLV1				I	2			G				I				I	0	2	G		home brew (2)				
CBFB INV(16)/MYH11				I				I	4			G	1	2		I	1	6	G/I				home brew (3)	home brew (1)	Vysis (3)
E2A-PBX t(1;19) (4;11)				I				I	1			I				I	0	1	I				home brew (1)		
MLL(11q23)/ AF4 (4;11)				I				I	3			G	1	2		I	1	5	G/I				home brew (3)		Vysis (3)
JAK 2 (V617F)				I	26			G	1			I				I	0	27	G	home brew (17), IVS(4), Ipsogen(2)	home brew(2), Eragen(1), Invader(1)	home brew (1)	home brew (1)		
JAK 2 (Exon 12)				I				I	1			I				I	0	1	I				home brew (1)	home brew (1)	
MPL W 515				I	2			G	1			I				I	0	3	G		NI (1), Invader(1)		home brew (1)		
MPL S 505				I				I	1			I				I	0	1	I				home brew (1)		
FLT 3 ITD				I	14			G				I				I	0	14	G		home brew (10), IVS(3), Seegene(1)				
FLT 3 D835				I	12			G				I				I	0	12	G		home brew (8), IVS(3), Seegene(1)				
NPM1 mutation				I	9			G				I				I	0	9	G		home brew (9)				
P53				I	2			R				I	2			G	2	2	I		home brew(2)				Vysis (2)
IgVH mutation				I	4			R	4			R				I	8	0	R	home brew(2), IVS(1)	IVS(1)	home brew (3), IVS (1)			
c-kit				I	3			G				I				I	0	3	G		home brew(3)				
Other:(MYC,RARA,ALK, Bcl-6), (HFE, RB1, etc)				I	1			I		1	1	I	1	2		I	1	2	I						Vysis (2)

**Table 3: New York State Molecular Oncology Proficiency Test -Summary of Results**

**Sample: NYS# L/L 3 (October 2008)**

Overall diagnosis: B-cell clone with IGH and IGK rearrangements, EBV positive, and IgVH mutation																										
Assay	SB				PCR				RT-PCR				FISH				All methods			Method used						
	R	G	ind	Cons	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)	FISH	
IGH	2			R	25			R				I	2			G	27	2	R/R/G	Dako (1), home brew (1)	Biomed (10), home brew (12), IVS (4)				Vysis (2)	
IGK	1			I	6			R				I				I	7	0	R	home brew (1)	Biomed (6)					
IGL		1		I				I				I				I	0	1	I	home brew (1)						
TRB	2	1		I	2	4		I				I				I	4	5	I	home brew (2), Dako (1)	Biomed (5), home brew (1), IVS (1)					
TRG				I	25			G				I				I	0	25	G		Biomed (5), home brew (17), IVS (2), NI (1)					
TRD		1		I				I				I				I	0	1	I	home brew (1)						
IGH/BCL2	MBR	1		I	13			G				I				I	0	14	G	home brew (1)	Biomed (2), IVS (1), home brew (8)	home brew (2)				
	mcr			I	11			G				I				I	0	11	G		home brew (2)					
	MBR 3'			I	2			G				I				I	0	2	G							
	MBR/mcr			I				I				I	3			G	0	3	G						Vysis (3)	
IGH/CCND1 (Bcl-1)		1		I	6			G				I	3			G	0	10	G	home brew (1)	home brew (3), other (1)	home brew (2)			Vysis (3)	
BCR/ABL1	p210		1		I			I	25			G				I	0	26	G	home brew (1)			home brew (7)	home brew (16), Ipsogen(3), Cagheid(1)		
	p190				I			I	21			G				I	0	21	G				home brew (9), Ipsogen(1)	home brew (12), Ipsogen(1)		
	p210/190				I			I	7			G	3			G	0	10	G				home brew (1), Roche(1)	home brew (3), Roche(2)	Vysis (3)	
	Abl kinase domain mutation				I			I	2			G				I	0	2	G				NI (3)			
PML/RARA	Long				I			I	12			G				I	0	12	G				home brew (6), Ipsogen(1)	home brew (5)		
	Short				I			I	10			G				I	0	10	G				home brew (5)	home brew (5)		
	Variable				I			I	4			G				I	0	4	G				home brew (3)	home brew (1)		
	Long/Short/Variable				I			I	1			I	3			G	0	4	G				home brew (1)		Vysis (3)	
MYC t(8;14)				I			I				I	3			G	0	3	G							Vysis (3)	
AML1/ETO t(8;21)				I			I	7			G	3			G	0	10	G				home brew (5)	home brew (2)	Vysis (3)		
NPM/ALK t(2;5)				I			I	1			I	1			I	0	2	G				home brew (1)		Vysis (1)		
IGH/BCL-6				I			I				I	2			G	0	2	G							Vysis (2)	
ETV6/RUNX1 (Tel-AML1)				I			I	3			G	2			G	0	5	G				home brew (2)	home brew (1)	Vysis (2)		
EBV				I	4			R			I				I	4	0	R		home brew (2)	Roche (1), other (1)					
KSHV/HHV8				I	3			G			I				I	0	3	G		home brew (3)						
HTLV1				I	2			G			I				I	0	2	G		home brew (2)						
CBFB INV(16)/MYH11				I			I	4			G	3			G	0	7	G				home brew (3)	home brew (1)	Vysis (3)		
E2A-PBX t(1;19) (4;11)				I			I	1			I				I	0	1	I				home brew (1)				
MLL(11q23)/ AF4 (4;11)				I			I	3			G	3			G	0	6	G				home brew (3)		Vysis (3)		
JAK 2 (V617F)				I	26			G	1		I				I	0	27	G		home brew (17), IVS(4), Ipsogen(2)	home brew(4), Eragen(1), Invader(1)	home brew (1)	home brew (1)			
JAK 2 (Exon 12)				I			I	1			I				I	0	1	I				home brew (1)	home brew (1)			
MPL W 515				I	2			G	1		I				I	0	3	G		HB (1), Invader(1)		home brew (1)				
MPL S 505				I			I	1			I				I	0	1	I				home brew (1)				
FLT 3 ITD				I	14			G			I				I	0	14	G		home brew (10), IVS(3), Seegene(1)						
FLT 3 D835				I	12			G			I				I	0	12	G		home brew (8), IVS(3), Seegene(1)	home brew (9)					
NPM1 mutation				I	9			G			I				I	0	9	G								
P53				I	2			G			I	2			G	0	4	G		home brew(2)					Vysis (2)	
IgVH mutation				I	4			R	3	1*	R				I	7	1*	R		home brew(2), IVS(1)	IVS(1)	home brew (3), IVS (1)				
c-kit				I	3			G			I				I	0	3	G		home brew(3)						
Other:(MYC,RARA,ALK, Bcl-6), (HFE, RB1, etc)				I	1			I			I	1	1		I	1	2	I		NI (1)					Vysis (2)	

\* No clonal band detected

**Table 4: Summary for IGH primer mix**

Reagent Source	Mix	L/L1			L/L2		L/L3		
		R	G	I*	R	G	R	G	Fail
BIOMED-2	A		8		8		8		
	B		8	1	9		9		
	C		8		8		8		
	D		2		2			2	
	E	1	2			3			3
IVS	FR 1		3		3		3		
	FR 2		4		4		2	1	1
	FR 3		4		4		4		
HOMEBREW	FR 1		3		3		3		
	FR 2		9		9		7	2	
	FR 3		12		11	1	12		

\*Indeterminate

**Table 5: Summary for IGK primer mix**

Reagent Source	Mix	L/L1		L/L2		L/L3	
		R	G	R	G	R	G
BIOMED-2	A		6	6		6	
	B		6	6		6	
IVS	A						
	B						
HOMEBREW	A						
	B						

**Table 6: Summary for TRG primer mix**

Reagent Source	Mix	L/L1		L/L2		L/L3		I*
		R	G	R	G	R	G	
BIOMED-2	A	5			5		5	
	B	5			5		5	
IVS	Mix 1	1	1		2		2	
	Mix 2		1		1		1	
HOMEBREW	V1-4							1
	V1-8	1	7		8		7	
	V9	8			8		8	
	V10	4			4		4	
	V11	1	4		5		5	
	V10,11	1			1		1	
	Mix 1 <sup>1)</sup>	2	1		2		2	
	Mix 2 <sup>1)</sup>	1	2		2		2	
	A <sup>2)</sup>	1			1		1	
B <sup>2)</sup>	1			1		1		

\*Indeterminate

<sup>1)</sup> Presumably IVS, but was not specified

<sup>2)</sup> Presumably Biomed-2, but was not specified

**Table 7: Summary for TRB primer mix**

Reagent Source	Mix	L/L1		L/L2		L/L3	
		R	G	R	G	R	G
BIOMED-2	A	3	2	1*	4		5
	B		5		5		5
	C	2	3		5	4	1
HOMEBREW	A	1			1		1
	B		1		1		1

\* Intensity below the 10% sensitivity control

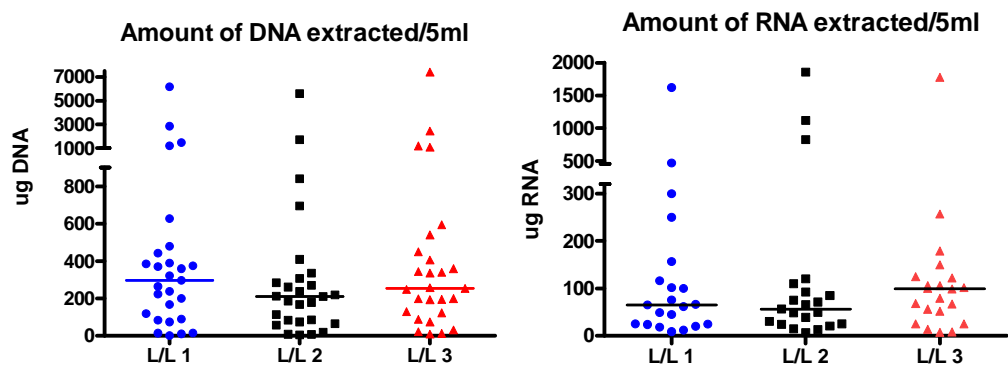


Figure 1. NYS PT MCTM 10-08 DNA and RNA yields

	LL1	LL2	LL3		LL1	LL2	LL3
Yield (ug)	DNA	DNA	DNA		RNA	RNA	RNA
Median	297	210.0	254.0		65.3	56.1	99
Max	6156	5580	7392		1623	1860	1781
Min	1.5	5.3	9.8		8.55	7	7.38