STATE OF NEW YORK
DEPARTMENT OF HEALTH

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Commissioner

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Molecular and Cellular Tumor Marker Proficiency Test Program
MCTM 11-10
Summary of results[1]

December 21, 2010

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from November 3, 2010, MCTM 11-10.

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 1). **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

[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

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' 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 31 laboratories that used PCR reported a rearrangement, as did the two laboratories that used SB. Rearrangements were near uniformly detected with the Biomed-2 tubes A and C that target the FR1 and FR3 regions, and the IVS or lab developed primers for the FR 1 and 3 regions (Table 4). In contrast, rearrangements were only detected by 50% (14/14) of the labs with primers against FR 2 (Biomed-2 tube B, IVS or lab developed). All twelve labs that tested for IGK (PCR=11, SB=1) reported a rearrangement; rearrangements were detected with both Biomed-2 tubes A and B, which target the Vk/Jk and Vk/Kde regions (Table 5). No translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any primers. Thus, these results suggest that this sample contained a B-cell clone with both IGH and IGK gene rearrangements.

T-cell tests: 27 out of 29 laboratories (93%) that tested for TRG by PCR found no rearrangement. Likewise, nine out of the eleven (82%) labs that tested for TRB by PCR found no rearrangement, as did the two labs that used SB. The two labs (18%) that reported a TRB rearrangement by PCR used the Biomed-2 tube C primers; one of these two labs also reported a rearrangement using the Biomed-2 tube B primers (Table 7). Furthermore, this lab reported a rearrangement for TRG using lab developed Vγ9 primers. Since both of these results are against the consensus (Table 6), this lab should check its assay performance and/or result interpretation. Overall however, there was a consensus that this sample did not contain cells with T-cell receptor rearrangements.

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

IGHV mutation: Eight labs tested for IGHV hypermutation (PCR=4, RT-PCR=4), and all concluded that this sample belonged to the IGHV3-13 family, but was not hypermutated.

The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, WT1, CEBPA, and RAS.

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

NYS#L/L 2 (Table 2):
B-cell tests: For IGH, 26 out of 30 (87%) laboratories reported no rearrangement by PCR. Of the four labs that did find a rearrangement, one reported results from two different primer sets, one lab developed, one Biomed-2, and found a rearrangement with both. Of the two labs that used SB one reported a rearrangement and the other reported an indeterminate result; therefore, there was no consensus for SB. Nine out of the eleven (82%) labs that tested for IgKappa (IGK) by PCR found no rearrangement, as did the one laboratory that tested for IGK by SB. No translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any primers. Thus, there was a consensus that this sample did not contain cells with immunoglobulin gene rearrangements.

T-cell tests: 25 out of 29 (86%) laboratories that tested for TcRGamma (TRG) by PCR found a rearrangement, possibly involving the Vγ9 and Vγ10 regions (Table 6). Of the four labs that did not find a TRG rearrangement, three used lab developed primers and one used Biomed-2 primers. Six out of the eleven (55%) labs that tested for TcRBeta (TRB) reported a rearrangement by PCR; however, the two labs that used SB did not detect a TRB rearrangement. Therefore, there was no 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. Thus, these results suggest that this sample contained a T-cell clone with T-cell receptor gamma gene rearrangement only.

The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, WT1, CEBPA, and RAS.

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 the presence of an early T-cell population.

NYS#L/L 3 (Table 3):
B-cell tests: For IGH, all 31 laboratories that used PCR reported a rearrangement, as did the two laboratories that used SB. Rearrangements were detected with the Biomed-2 tubes A and B, which target the FR1 and FR2 regions, but generally not tube C, which targets FR3 (Table 4). Interestingly, however, rearrangements were detected in all three frameworks by the IVS primers, and framework 2 and 3 by lab developed primers (Table 4). All twelve labs that tested for IGK (PCR=11, SB=1) reported a rearrangement, possibly involving the Kde region detected by the Biomed-2 tube B (nine out of ten labs 90%, Table 5), whereas only half the labs also detected a rearrangement involving the Vk/Jk regions detected by the Biomed-2 tube A. No translocations involving IGH/BCL2 MBR (except one lab using the Biomed-2 primers), mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any primers. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: 25 out of 29 laboratories (86%) that tested for TRG by PCR found no rearrangement, whereas three labs reported a rearrangement and one result was indeterminate. All thirteen labs that tested for TRB (PCR=11, SB=2) found no rearrangement. Thus, there was a consensus that this sample did not contain cells with T-cell receptor rearrangements.

IGHV mutation: Of the eight labs that tested for IGHV hypermutation (PCR=4, RT-PCR=4), five reported mutation rates between 8.3-9.9%, and assigned the sequence to the IGHV3-23 family, whereas one did not

report the mutation rate and family. In contrast, one lab did not detect a clonal band, and the other lab reported an indeterminate result.

RAS: One lab that tested for RAS reported a K-RAS mutation (G13D) by PCR and sequencing.

The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, WT1 tumor, and CEBPA.

In aggregate, these results indicate that the sample contained a B-cell clone possibly with IGHV hypermutation. This conclusion is in agreement with the result from Flow Cytometry, which also indicated the presence of an early immature B-cell population.

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 4.7, 4.5, and 4.1 µg per 5 ml specimen to a maximum of 73,900, 126,000, and 29,500 µg, respectively, corresponding to a 7,195 to 28,000-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 2.5, 2.9, and 2.7 µg to 2,820, 2,620, and 2,395 µg, respectively, corresponding to an 887- to 1,128-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, and your units are in microgram (µg), not nanogram (ng) or milligram (mg). Presumably, differences in the methods used for DNA and RNA isolation also contributed to this wide range. However, it also raises the question of how accurate some of the measurements are. The one lab that reported L/L1, 2, and 3 DNA yields high as 73,900, 126,000 and 29,500 µg, should check its DNA measurement method; these results were not included in Figure 1. 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 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 home brew 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**
February 28	March 29
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 11 2010.xls

Interpretation:	B-cell clone with IGH and IGK gene rearrangements and presence of EBV sequence																			
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	31			R					33	0	R	Dako (1), home brew (1)	Biomed (12), home brew (16), IVS (5)			
IGK	1			I	11			R					12	0	R	home brew (1)	Biomed (9), home brew (2)			
IGL													0	0						
TRB		2		I	2	9		G					2	11	G	home brew (1), Dako (1)	Biomed (8), home brew (3),			
TRG					2	27		G					2	27	G		Biomed (10), home brew (16), IVS (3),			
TRD													0	0						
IGH/BCL2 MBR						15		G					0	15	G		Biomed (3), IVS (3), home brew (7)	home brew (2)		
mcr						12		G					0	12	G		Biomed (3), IVS (2), home brew (5)	home brew (2)		
MBR 3'						3		G					0	3	G		Biomed (3)			
MBR/mcr													0	0						
IGH/CCND1 (Bcl-1)						8		G					0	8	G		home brew (3), Biomed (2), IVS (1)	home brew (2)		
BCR/ABL1 p210										25		G	0	25	G				home brew (5)	home brew (16), Ipsogen(6), Cepheid(1)
p190										21		G	0	21	G				home brew (7)	home brew (11), Ipsogen(6)
p210/190										6		G	0	6	G				home brew (2)	home brew (1), Roche(3), 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(2)
Short										12		G	0	12	G				home brew (5)	home brew (6) Ipsogen(2)
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)										4		G	0	4	G				home brew (2)	home brew (2) other(1)
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										1		I	0	1	I				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 (5) Ipsogen(1)		home brew (3)	home brew (1)
MPL W 515						5		G		2		I	0	7	G		home brew (4)	Invader(1)	home brew (2)	
MPL S 505						3		G		2		I	0	5	G		home brew (3)		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						8		G					0	8	G		home brew (7)			
P53						2		I					0	2	I		home brew(2)			
IGHV mutation						4		G		4		G	0	8	G		home brew(3)	home brew(1)	home brew (3) IVS (1)	
c-kit						7		G					0	7	G		home brew(7)			
Other ‡						1		I						1	I		home brew(1)			

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 11 2010.xls

Interpretation:	T-cell clone with TRG gene rearrangement																			
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		1	I	4	26		G					5	26	I/G	Dako (1), home brew (1)	Biomed (11), home brew (15), IVS (5)			
IGK		1		I	2	9		G					2	10	G	home brew (1)	Biomed (9), home brew (2)			
IGL													0	0						
TRB		2		I	6	5		I					6	7	I	home brew (1), Dako (1)	Biomed (8), home brew (3),			
TRG					25	4		R					25	4	R		Biomed (10), home brew (16), IVS (3),			
TRD													0	0						
IGH/BCL2 MBR						15		G					0	15	G		Biomed (3), IVS (3), home brew (7)	home brew (2)		
mcr						12		G					0	12	G		Biomed (3), IVS (2), home brew (5)	home brew (2)		
MBR 3'						3		G					0	3	G		Biomed (3)			
MBR/mcr													0	0						
IGH/CCND1 (Bcl-1)						8		G					0	8	G		home brew (3), Biomed (2), IVS (1)	home brew (2)		
BCR/ABL1 p210										25		G	0	25	G				home brew (5)	home brew (16), Ipsogen(6), Cepheid(1)
p190										21		G	0	21	G				home brew (7)	home brew (11), Ipsogen(6)
p210/190										6		G	0	6	G				home brew (2)	home brew (1), Roche(3), 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(2)
Short										12		G	0	12	G				home brew (5)	home brew (6) Ipsogen(2)
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)										4		G	0	4	G				home brew (2)	home brew (2) other(1)
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					1	2		I					1	2	I		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										1		I	0	1	I				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 (5) Ipsogen(1)		home brew (3)	home brew (1)
MPL W 515						5		G		2		I	0	7	G		home brew (4)	Invader(1)	home brew (2)	
MPL S 505						3		G		2		I	0	5	G		home brew (3)		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						8		G					0	8	G		home brew (7)			
P53						2		I					0	2	I		home brew(2)			
IGHV mutation						4N		N		3N		N	0	7N	N		home brew(3)	home brew(1)	home brew (3) IVS (1)	
c-kit						7		G					0	7	G		home brew(7)			
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 11 2010.xls

Interpretation: B-cell clone with IGH and IGK gene rearrangements and IGHV hypermutation

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	31			R					33	0	R	Dako (1), home brew (1)	Biomed (12), home brew (16), IVS (5)			
IGK	1			I	11			R					12	0	R	home brew (1)	Biomed (9), home brew (2)			
IGL													0	0						
TRB		2		I		11		G					0	13	G	home brew (1), Dako (1)	Biomed (8), home brew (3),			
TRG					3	25	1	G					3	25	G		Biomed (10), home brew (16), IVS (3),			
TRD													0	0						
IGH/BCL2 MBR					1	14		G					1	14	G		Biomed (3), IVS (3), home brew (7)	home brew (2)		
mcr						12		G					0	12	G		Biomed (3), IVS (2), home brew (5)	home brew (2)		
MBR 3'						3		G					0	3	G		Biomed (3)			
MBR/mcr													0	0						
IGH/CCND1 (Bcl-1)						8		G					0	8	G		home brew (3), Biomed (2), IVS (1)	home brew (2)		
BCR/ABL1 p210										25		G	0	25	G				home brew (5)	home brew (16), Ipsogen(6), Cepheid(1)
p190										21		G	0	21	G				home brew (7)	home brew (11), Ipsogen(6)
p210/190										6		G	0	6	G				home brew (2)	home brew (1), Roche(3), 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(2)
Short										12		G	0	12	G				home brew (5)	home brew (6) Ipsogen(2)
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)										4		G	0	4	G				home brew (2)	home brew (2) other(1)
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										1		I	0	1	I				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 (5) Ipsogen(1)		home brew (3)	home brew (1)
MPL W 515						5		G		2		I	0	7	G		home brew (4)	Invader(1)	home brew (2)	
MPL S 505						3		G		2		I	0	5	G		home brew (3)		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						8		G					0	8	G		home brew (7)			
P53						2		I					0	2	I		home brew(2)			
IGHV mutation					4			R	2	1N	1	I	6	1N	R		home brew(3)	home brew(1)	home brew (3) IVS (1)	
c-kit						7		G					0	7	G		home brew(7)			
Other ‡					1	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	10		R	1	7	G	10		R
	B	5	6	I	1	8	G	11		R
	C	10	1	R	2	8	G	2	9	G
	D		3	G		3	G		3	G
	E		4	G		4	G		4	G
IVS	FR 1	4		R		4	G	4		R
	FR 2	4	2	I	1	5	G	6		R
	FR 3	5	1	R	1	5	G	4	2	I
Lab developed	FR 1	2		I		2	I	1	1	I
	FR 2	5	6	I	1	9	G	11		R
	FR 3	13	1	R		13	G	10	4	R

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	11		R		11	G	5	5	I
	B	11		R	2	9	G	9	1	R

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		10	G	9	1	R	1	9	G
	B	1	9	G	9	1	R	3	6	I
IVS	Mix 1		9	G	7	2	R		9	G
	Mix 2		8	G	4	4	I		8	G
Lab developed	Vγ1-8		7	G	1	6	G		6	G
	Vγ9	1	5	G	4	2	I		5	G
	Vγ10		6	G	4	2	I		6	G
	Vγ11		3	G		3	G		3	G

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		8	G	4	4	I		8	G
	B	1	7	G	1	7	G		8	G
	C	2	6	G		8	G		8	G
Lab developed	A		2	I	2		I		2	I
	B		2	I		2	I		2	I

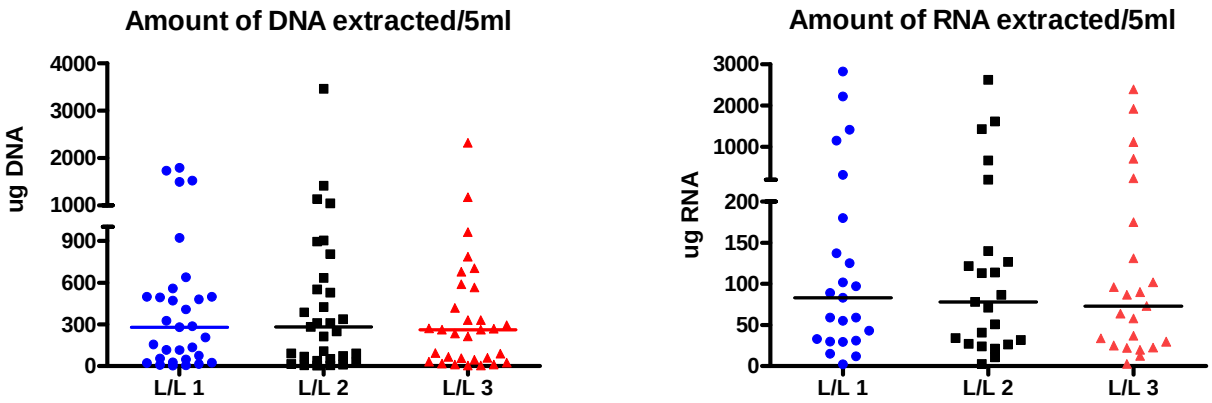

Figure 1. NYS MCTM PT 11-10 DNA and RNA yields

	LL1	LL2	LL3		LL1	LL2	LL3
Yield (ug)	DNA	DNA	DNA		RNA	RNA	RNA
Median	284	296.0	262.0		83.0	78	73.2
Max	73900	126000	29500		2820	2620	2395
Min	4.7	4.5	4.1		2.5	2.9	2.7

STATE OF NEW YORK
DEPARTMENT OF HEALTH

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Molecular Oncology Proficiency Test - MCTM 11-10
Summary of Results of FISH Testing

Participating laboratories were asked to test the three proficiency test samples by FISH for abnormalities in 20q12, ATM, BCL6, BCR/ABL, IGH/MYC, and MLL. Six of the seven participating laboratories performed all requested testing, while one laboratory reported results for a MYC breakapart test instead of IGH/MYC. All laboratories reporting IGH/MYC results included a CEP8 probe in the test and three laboratories included a P53 probe with the ATM probe. The assays used are listed in the table below.

Assay Target	Vendor	Product Number	Probe Description
20q12 (D20S108)	Abbott Molecular Inc	05J47-011	LSI, single color
ATM		05J83-001	LSI, multi-color, with P53, D13S319, 13q14, CEP12
ATM		05J64-001	LSI, single color
BCL6		01N23-020	LSI, dual color breakapart
BCR/ABL		05J82-001	Dual color, dual fusion
BCR/ABL		05J79-001	Dual color, extra signal BCR, ABL with 9q34 (ASS)
IGH/MYC		05J75-001	Tri-color, dual fusion, with CEP8
MLL		05J90-001	LSI, dual color breakapart

The reported results are summarized in more detail below and in the attached tables. Each table lists the range of abnormal results reported for each assay and the result reported by your laboratory; normal results are not presented in the summaries. The **"# labs concordant/ #labs testing"** column gives the fraction of labs that were concordant for that abnormal result.

P53 and CEP8 results were consistent with those of the other probes in their respective hybridizations and are not reported here. At least three labs reported results for each of the tests, therefore the results were considered evaluable for each test and were scored.

For copy number aberrations, the result reported by each laboratory was compared to the range reported by the other participating laboratories for both copy number per cell and the fraction of cells that were abnormal. Copy number aberrations were judged to be acceptable if the range or copy number and the frequency of cells reported were concordant with results from other participating laboratories. Abnormal results reported by a single laboratory but at a low frequency in the sample were also judged to be concordant. There were only two rearrangements reported. A BCR/ABL rearrangement was reported in a small fraction of cells by a single laboratory, as noted below for sample 1. Also, the laboratory performing a MYC breakapart test reported rearrangement in sample 3.

In addition to this results summary, each lab separately received a "sample score" for each sample based on the fraction of evaluable assays that gave results concordant with the other participating laboratories' results. Each lab also received an "assay score" for each assay based on the fraction of the three samples for which the lab's results were concordant with the other participating laboratories' results. Please keep in mind that this was an educational PT only.

Sample-specific comments:
- Sample L/L1:
All laboratories reported the presence of normal cells as well as a large fraction of cells with copy number gains for each of the six tests. In addition, one laboratory reported a low frequency of cells with a BCR/ABL rearrangement and loss of one fusion ((ABL,BCR)X2,(ABL con BCRx1)[2/200]). Given the low frequency, other participating laboratories were not penalized for not reporting this rearrangement. Furthermore, no bcr/abl fusion product was detected by RT-PCR, raising the question whether the underlying fusion was indeed present.

- Sample L/L2:
All laboratories reported either no abnormal cells or a very small fraction of abnormal cells for each of the six tests. One laboratory reported a low frequency of cells with a deletion of 20q12 (3/200 cells). Given the low frequency, other participating laboratories were not penalized for not reporting this deletion. No rearrangements were reported.

- Sample L/L3:
All laboratories reported the presence of normal cells as well as a large fraction of cells with copy number gains for each of the six tests. In addition, the laboratory that performed the MYC breakapart assay instead of the IGH/MYC assay reported a high frequency of cells with a MYC rearrangement (165/200 cells).

Comments on ISCN nomenclature:
All results for cancer samples should include the number of cells analyzed.
Results for breakapart or fusion probes must include the number of signals for each part of the fusion (e.g. "5'MLL con 3'MLLx3").

NEW YORK STATE MOLECULAR ONCOLOGY PROFICIENCY TEST - MCTM 11-10

		Abnormal FISH Results - All Reporting Laboratories					
Target Locus/Loci	Signal Pattern Normal/ Abnormal?	Number of Cells Analyzed	MINIMUM Frequency of Cells With This Signal Pattern	MAXIMUM Frequency of Cells With This Signal Pattern	RANGE OF NUMBER of Fusions Per Cell	RANGE OF NUMBER of Non-Fusion Signals Per Cell	#labs concordant/ #labs testing
Sample L/L-1							
20q12 (D20S108)	abnormal	200, 300	0.59	0.90	NA	3~7x	7/7
ATM	abnormal	200, 300	0.34	0.92	NA	3~6x	7/7
BCL6	abnormal	200, 300	0.62	0.88	3~7x	0	7/7
BCR/ABL	abnormal	200, 300	0.74	0.95	0	3~5x/3~5x	7/7
IGH/MYC	abnormal	200, 300	0.64	0.93	0	3~6x/4~6x	6/6
MLL	abnormal	200, 300	0.60	0.99	3~6x	0	7/7
Sample L/L-2							
20q12 (D20S108)	abnormal	200, 300	0.01	0.07	NA	4x	7/7
ATM	abnormal	200, 300	0.01	0.01	NA	4x	7/7
BCL6	abnormal	200, 300	0.01	0.01	4x	0	7/7
BCR/ABL	abnormal	200, 300	0.01	0.05	0	4x/4x	7/7
IGH/MYC	abnormal	200, 300	0.01	0.01	0	4x/4x	6/6
MLL	abnormal	200, 300	no abnormal results reported				7/7
Sample L/L-3							
20q12 (D20S108)	abnormal	200, 300	0.07	0.18	NA	3-6x	7/7
ATM	abnormal	200, 300	0.03	0.17	NA	3-4x	7/7
BCL6	abnormal	200, 300	0.05	0.13	4x	0	7/7
BCR/ABL	abnormal	200, 300	0.06	0.15	0	4x/4x	7/7
IGH/MYC	abnormal	200, 300	0.11	0.16	0	4x/4x	6/6
MLL	abnormal	200, 300	0.06	0.17	3-4x	0	7/7