NEW YORK
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HEALTH

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Commissioner

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Executive Deputy Commissioner

Molecular and Cellular Tumor Marker Proficiency Test Program
MCTM 6-11
Summary of results[1]

August 26, 2011

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from June 28, 2011, MCTM 6-11.

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**. **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 ≥80% 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 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 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

[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

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 2011-04 (Table 1):
B-cell tests: For IGH, all 29 laboratories that used PCR reported a rearrangement, as did the two laboratories that used SB. Rearrangements were 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 30-60% of the labs with primers against FR 2 (Biomed-2 tube B, IVS or lab developed). All nine labs that tested for IGK by PCR 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: all 27 laboratories that tested for TRG by PCR found no rearrangement. Likewise, all eleven labs that tested for TRB by PCR found no rearrangement, as did the two labs that used SB. Thus, there was a consensus that this sample did not contain cells with T-cell receptor rearrangements.

EBV: Three out of four labs (75%) that tested for EBV detected the presence of EBV sequences by PCR.

IGHV mutation: Ten labs tested for IGHV hypermutation (PCR=6, 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, that are not shown in the summary table.

In aggregate, these results indicate that the sample contained a B-cell clone containing EBV sequences.

NYS#L/L 2011-05 (Table 2):
B-cell tests: For IGH, 24 out of 28 laboratories (86%) that used PCR reported no rearrangement. Of the four labs that reported a rearrangement three used the Biomed-2 tube D that detects rearrangements in the DH regions 1-6, whereas one lab reported a rearrangement with a lab developed FR3 primer mix. Thus, these labs concluded that this sample was IGH rearranged a conclusion that is supported by the positive Southern blot result. All ten 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 5). All but one laboratory that tested for IGH/BCL2 by various methods reported no translocation, with the one exception reporting a translocation in the major breakpoint region. Similarly, none of the four laboratories that tested for the IGH/CCND1 (Bcl-1) by any method found a translocation. Thus, the consensus was that this sample contained cells with an immunoglobulin kappa gene rearrangement and possibly a somewhat rare rearrangement in the DH region of the IGH gene.

T-cell tests: 26 out of 27 laboratories (96%) that tested for TRG by PCR found no rearrangement. Likewise, all thirteen labs that tested for TRB reported no rearrangement (SB=2, PCR=11). These results suggest that this sample did not contain cells with T-cell receptor gene rearrangements.

EBV: Three out of four labs (75%) that tested for EBV detected the presence of EBV sequences by PCR.

The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, WT1, CEBPA, and RAS, that are not shown in the summary table.

In aggregate, these results indicate that the sample contained a clonal B-cell population.

NYS#L/L 6 (Table 3):
B-cell tests: For IGH, all 29 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 all IVS and lab developed primers against FR1 and 3 (Table 4); in contrast, results with IVS and lab developed primers targeting FR2 were mixed, with 4/5 IVS and 2/8 lab developed primers not detecting a rearrangement, suggesting different primer pairs in these FR2 mixtures compared to the Biomed-2 tube B mixture. All nine labs that tested for IGK by PCR reported a rearrangement with both Biomed-2 tubes A and B primers (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: 26 out of 27 laboratories (96%) that tested for TRG by PCR found no rearrangement. Six out of eleven (55%) labs that tested for TRB by PCR also indicated no rearrangement, in agreement with results from SB performed by two labs. The five (45%) labs that detected a possible TRB gene rearrangement based their interpretation on a sole positive result from the Biomed-2 tube C, whereas one lab, though finding tube C to be positive, did not report the sample as TRB positive because it concluded that the clinical significance of a positive tube C result alone is unclear. This lab suggested that tube C detects an incomplete (D-J) TCR beta gene rearrangement that usually has a poor correlation with the neoplastic process in the T-cell lineage. Thus, these results suggest that this sample may not have contained cells with a clinically relevant T-cell receptor gene rearrangement. A similar conclusion regarding a positive incomplete (D-J) TRB rearrangement result alone was reported by Dictor et al. Haematologica 90; 1524, 2005.

EBV: Three out of four labs (75%) that tested for EBV detected the presence of EBV sequences by PCR.

IGHV mutation: Eight out of ten (80%) labs reported IGHV hypermutation. All labs assigned the mutations to the IGHV1-46 family, and seven reported mutation rates between 8.65 to 10.68%, while one lab did not specify the mutation rate. One lab failed to detect a clonal band with its lab developed primers, suggesting a problem with its PCR, whereas another lab reported an indeterminate result.

The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, WT1, CEBPA, and RAS, that are not shown in the summary table.

In aggregate, these results indicate that the sample contained a B-cell clone with IGHV hypermutation and the presence of EBV virus sequence.

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). Furthermore, Tables 8-10 show a summary of 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/L4, 5, and 6 ranged from a minimum of 0.3, 1.0, and 2.0 µg/ml to a maximum of 270, 341, and 290 µg/ml, respectively, corresponding to a 145- to 900-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L4, 5 and 6 also ranged broadly from 1.0, 1.0, and 1.0 µg/ml to 258, 332, and 378 µg/ml, respectively, corresponding to a 258- to 378-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. 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 date for the next Molecular and Cellular Tumor Marker PT mail-out in 2011 is:

Mail-out date	**Due Date**
October 25	November 23

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/L 2011-04 Consensus Summaries PT 6-11

Interpretation:	B-cell clone with IGH and IGK gene rearrangements and presence of EBV														
Assay	SB				PCR				RT-PCR				All methods		
	R	G	ind	Cons[#]	R	G	ind	Cons[#]	R	G	ind	Cons[#]	R	G	Cons[#]
IGH	2			I	29			R					31	0	R
IGK					9			R					9	0	R
IGL															
TRB		2		I		11		G					0	13	G
TRG						27		G					0	27	G
TRD															
IGH/BCL2 MBR						11		G					0	11	G
mcr						8		G					0	8	G
MBR 3'						3		G					0	3	G
IGH/CCND1 (Bcl-1)						4		G					0	4	G
BCR/ABL1 p210										27		G	0	27	G
p190										25		G	0	25	G
p210/190										10		G	0	10	G
Abl kinase domain mutation										2		I	0	2	I
PML/RARA Long										12		G	0	12	G
Short										11		G	0	11	G
Variable										3		G	0	3	G
MYC t(8;14)															
AML1/ETO t(8;21)										5		G	0	5	G
NPM/ALK t(2;5)															
IGH/BCL-6															
ETV6/RUNX1 (Tel-AML1)										2		I	0	2	I
EBV					3	1		I					3	1	I
KSHV/HHV8						3		G					0	3	G
HTLV1						2		I					0	2	I
CBFB INV(16)/MYH11										2		I	0	2	I
E2A-PBX t(1;19) (4;11)										1		I	0	1	I
MLL(11q23)/ AF4 (4;11)										2		I	0	2	I
JAK 2 (V617F)						26		G		2		I	0	28	G
JAK 2 (Exon 12)						5		G		3		G	0	8	G
MPL W 515						6		G		2		I	0	8	G
MPL S 505						4		G		2		I	0	6	G
FLT 3 ITD						10		G					0	10	G
FLT 3 D835						9		G					0	9	G
NPM1 mutation						12		G					0	12	G
P53					1	1		I					1	1	I
IGHV mutation						6		G		4		G	0	10	G
c-kit						8		G					0	8	G
Other [‡]															

Cons [#]: R or G based on ≥80% consensus; I if <80% consensus or <3 results

Other [‡] : See critique for details.

Table 2: New York State Molecular Oncology Proficiency Test
Sample: NYS# L/L 2011-05 Consensus Summaries PT 6-11

Interpretation:	B-cell clone with IGK gene rearrangement and presence of EBV (†but see discussion regarding minority result for IGH)														
Assay	SB				PCR				RT-PCR				All methods		
	R	G	ind	Cons#	R	G	ind	Cons#	R	G	ind	Cons#	R	G	Cons#
IGH	2			I	4†	24		G					6	24	G
IGK					10			R					10	0	R
IGL															
TRB		2		I		11		G					0	13	G
TRG					1	26		G					1	26	G
TRD															
IGH/BCL2 MBR					1	9		G					1	9	G
mcr						7		G					0	7	G
MBR 3'						3		G					0	3	G
IGH/CCND1 (Bcl-1)						4		G					0	4	G
BCR/ABL1 p210										28		G	0	28	G
p190										26		G	0	26	G
p210/190										10		G	0	10	G
Abl kinase domain mutation										2		I	0	2	I
PML/RARA Long										12		G	0	12	G
Short										11		G	0	11	G
Variable										3		G	0	3	G
MYC t(8;14)															
AML1/ETO t(8;21)										5		G	0	5	G
NPM/ALK t(2;5)															
IGH/BCL-6															
ETV6/RUNX1 (Tel-AML1)										2		I	0	2	I
EBV					3	1		I					3	1	I
KSHV/HHV8						3		G					0	3	G
HTLV1						2		I					0	2	I
CBFB INV(16)/MYH11										2		I	0	2	I
E2A-PBX t(1;19) (4;11)										1		I	0	1	I
MLL(11q23)/ AF4 (4;11)										2		I	0	2	I
JAK 2 (V617F)		1		I		25		G		2		I	0	28	G
JAK 2 (Exon 12)						5		G		3		G	0	8	G
MPL W 515						6		G		2		I	0	8	G
MPL S 505						4		G		2		I	0	6	G
FLT 3 ITD						10		G					0	10	G
FLT 3 D835						9		G					0	9	G
NPM1 mutation						12		G					0	12	G
P53						2		I					0	2	I
IGHV mutation						5N		N		3N	1	N	0	8N	N
c-kit						8		G					0	8	G
Other ‡															

N: No clonal band detected

Cons #: R or G based on ≥80% consensus; I if <80% consensus or <3 results

Other ‡ : See critique for details.

Table 3: New York State Molecular Oncology Proficiency Test
Sample: NYS# L/L 2011-06 Consensus Summaries PT 6-11

Interpretation:	B-cell clone with IGH and IGK gene rearrangements, IGHV hypermutation, and presence of EBV														
Assay	SB				PCR				RT-PCR				All methods		
	R	G	ind	Cons[#]	R	G	ind	Cons[#]	R	G	ind	Cons[#]	R	G	Cons[#]
IGH	2			I	29			R					31	0	R
IGK					9			R					9	0	R
IGL															
TRB		2		I	5	6		I					5	8	I
TRG					1	26		G					1	26	G
TRD															
IGH/BCL2 MBR						11		G					0	11	G
mcr						8		G					0	8	G
MBR 3'						3		G					0	3	G
IGH/CCND1 (Bcl-1)						4		G					0	4	G
BCR/ABL1 p210										28		G	0	28	G
p190										26		G	0	26	G
p210/190										10		G	0	10	G
Abl kinase domain mutation										2		I	0	2	I
PML/RARA Long										12		G	0	12	G
Short										11		G	0	11	G
Variable										3		G	0	3	G
MYC t(8;14)															
AML1/ETO t(8;21)										5		G	0	5	G
NPM/ALK t(2;5)															
IGH/BCL-6															
ETV6/RUNX1 (Tel-AML1)										2		I	0	2	I
EBV					3	1		I					3	1	I
KSHV/HHV8						3		G					0	3	G
HTLV1						2		I					0	2	I
CBFB INV(16)/MYH11										2		I	0	2	I
E2A-PBX t(1;19) (4;11)										1		I	0	1	I
MLL(11q23)/ AF4 (4;11)										2		I	0	2	I
JAK 2 (V617F)		1		I		25		G		2		I	0	28	G
JAK 2 (Exon 12)						5		G		3		G	0	8	G
MPL W 515						6		G		2		I	0	8	G
MPL S 505						4		G		2		I	0	6	G
FLT 3 ITD						10		G					0	10	G
FLT 3 D835						9		G					0	9	G
NPM1 mutation						12		G					0	12	G
P53						2		I					0	2	I
IGHV mutation					5			R	3	1N	1	I	8	1N	R
c-kit						8		G					0	8	G
Other [‡]															

N: No clonal band detected

Cons [#]: R or G based on ≥80% consensus; I if <80% consensus or <3 results

Other [‡]: See critique for details.

Individual Primers PT 6-11

Table 4: Summary for IGH primer mix

Reagent Source	Mix	L/L4		CONSENSUS	L/L5		CONSENSUS	L/L6		CONSENSUS
0	0	R	G		R	G		R	G	
Biomed-2	A	12	0	R	0	11	G	12	0	R
	B	4	8	I	0	12	G	13	0	R
	C	12	0	R	0	12	G	12	0	R
	D	0	3	G	3	0	R	0	3	G
	E	0	4	G	0	4	G	0	4	G
IVS	FR 1	4	0	R	0	4	G	4	0	R
	FR 2	3	2	I	0	4	G	1	4	G
	FR 3	6	0	R	0	5	G	6	0	R
Lab developed	FR 1	3	0	R	0	3	G	3	0	R
(home brew)	FR 2	4	4	I	0	8	G	6	2	I
	FR 3	12	0	R	1	11	G	11	0	R

Table 5: Summary for IGK primer mix

Reagent Source	Mix	L/L4		CONSENSUS	L/L5		CONSENSUS	L/L6		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A	7	0	R	8	0	R	7	0	R
	B	8	0	R	7	1	R	7	0	R
Lab developed	A	1	0	I	2	0	I	2	0	I
(home brew)	B	1	0	I	2	0	I	2	0	I

Table 6: Summary for TRG primer mix

Primer Source	Mix	L/L4		CONSENSUS	L/L5		CONSENSUS	L/L6		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A	0	12	G	0	12	G	0	12	G
	B	0	12	G	0	12	G	0	12	G
IVS	Mix 1	0	3	G	0	3	G	0	3	G
	Mix 2	0	3	G	0	3	G	0	3	G
Lab developed	Vγ1-8	0	6	G	0	6	G	0	6	G
(home brew)	Vγ9	0	5	G	1	4	G	1	4	G
	Vγ10	0	5	G	0	5	G	0	5	G
	Vγ11	0	4	G	0	4	G	0	4	G
	Mix 1	0	4	G	0	4	G	0	4	G
	Mix 2	0	4	G	0	4	G	0	4	G

Table 7: Summary for TRB primer mix

Primer Source	Mix	L/L4		CONSENSUS	L/L5		CONSENSUS	L/L6		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A	0	8	G	0	8	G	0	9	G
	B	0	8	G	0	8	G	0	9	G
	C	0	7	G	0	7	G	6	2	I
Lab developed	A	0	1	I	0	1	I	0	1	I
(home brew)	B	0	1	I	0	1	I	0	1	I

Method Summaries PT 6-11

Table 8

Analyte	Reagents / Method	Lab dev	Dako	IVS (Biomed2)	IVS (not Biomed2)
IGH	SB	2	1		1
	PCR	12	1	13	6
IGHV (IgVH)	PCR	3			3
	RT-PCR	4			
IGK	SB				
	PCR	1		8	
TRB	SB	1	1		
	PCR	2		9	
TRG	SB				
	PCR	12		12	3
IGH/BCL2 MBR	SB				
	PCR	6		4	
IGH/BCL2 mcr	SB				
	PCR	4		3	
IGH/BCL2 3'MBR	SB				
	PCR			3	
IGH/CCND1	SB				
	PCR	3			
	qPCR	1			

Table 9

Analyte	Reagents / Method	Lab dev	Ipsogen
JAK2 V617F	PCR	18	1
	qPCR	5	4
	RT-PCR	2	
	qRT-PCR	1	
JAK2 exon 12	PCR	4	
	qPCR	2	1
	RT-PCR	3	
	qRT-PCR	1	
MPL W515	PCR	5	
	RT-PCR	2	
MPL S505	PCR	3	
	RT-PCR	2	
FLT3 ITD	PCR	8	1
FLT3 D835	PCR	7	1
NPM1	PCR	12	
c-kit	SB		
	PCR	8	

Method Summaries PT 6-11

Table 10

Analyte	Reagents / Method	Lab dev	Asuragen	Cepheid	Ipsogen	Roche
BCR/ABL1 p210	RT-PCR	9		1	1	
	qRT-PCR	20	1	1	6	
BCR/ABL1 p190	RT-PCR	9		1	1	
	qRT-PCR	18	1	1	6	
BCR/ABL1 p210/p190	RT-PCR	3		1	1	
	qRT-PCR	6		1	2	3
PML/RARA long form	RT-PCR	5				
	qRT-PCR	6			1	
PML/RARA short form	RT-PCR	4				
	qRT-PCR	6			1	
PML/RARA variable form	RT-PCR	2				
	qRT-PCR				1	
AML1/ETO t(8;21)	RT-PCR	3				
	qRT-PCR	2				
ETV6/RUNX1 t(12;21)	RT-PCR	1				
	qRT-PCR	1				
CBFB INV(16) CBFB/MYH11	RT-PCR	1				
	qRT-PCR	1				
E2A-PBX t(1;19) E2A-PBX t(4;11)	RT-PCR	1				
	qRT-PCR					
MLL (11q23) MLL/AF4 (4;11)	RT-PCR	2				
	qRT-PCR					

Reference genes	bcr/abl	PML/RARA	AML1/ETO
Abl	17	4	2
G6PDH	4	0	
GUSB	2	2	
TBP	3	2	1
BCR	1	1	1
B2 microglobulin	2	0	0

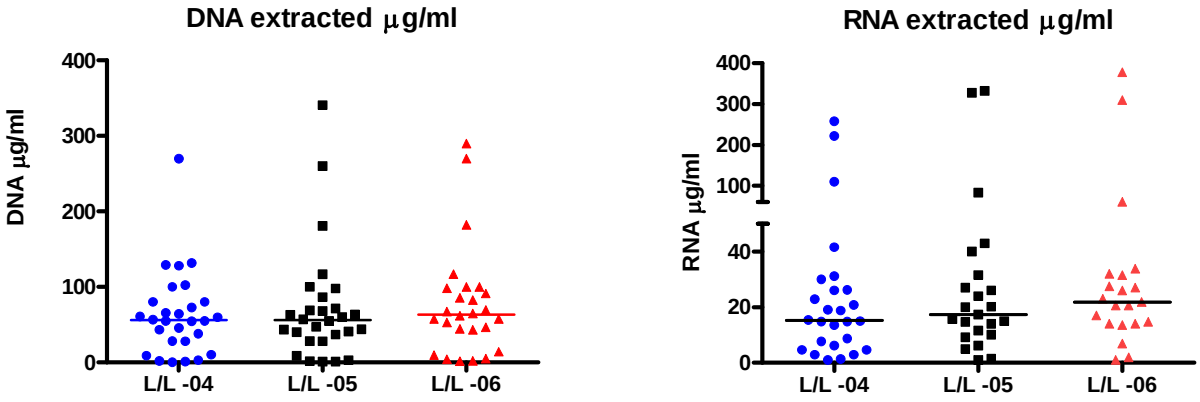


Figure 1. NYS MCTM PT 6-11 DNA and RNA yields. The yields were converted to ug DNA and RNA per 1 ml blood.

	L/L 2011-04	L/L 2011-05	L/L 2011-06		L/L 2011-04	L/L 2011-05	L/L 2011-06
	DNA	DNA	DNA		RNA	RNA	RNA
Median	55	47.3	66.1		15	17.3	20.6
Max	270	340.8	290		258	332	377.8
Min	0.3	1	2		1	1	1

New York State Oncology - Molecular and Cellular Proficiency Test - June, 2011
Participant Summary - FISH Testing
September 30, 2011

Below is a summary of interphase FISH results for the June, 2011 proficiency test event for Oncology - Molecular and Cellular Tumor Markers (ONCOMCTM). This summary includes a general overview of the event, sample-specific summaries, and comments on the use of the International System for Human Cytogenetic Nomenclature (ISCN). Enclosed are tables that describe the reported results for each sample in greater detail and a summary of your laboratory's performance for this part of the event.

Overview
Participating laboratories were asked to test three proficiency test samples using interphase FISH to rule out a clinical diagnosis. Eight laboratories received samples for FISH testing and each sample was tested by all laboratories. Each laboratory selected assays appropriate to the reason for referral and reported results for copy number and/or rearrangement of the target regions for these assays. Each laboratory also reported the results for each sample using ISCN nomenclature. In evaluating the results, the result reported by each laboratory was compared to the range reported by the other participating laboratories. If a laboratory's result for an assay fell within this range, that laboratory's result was determined to be "concordant" (acceptable). Your laboratory's results are described in the enclosed sample tables. In general, the results reported for each assay were quite consistent among the participants. Probes that targeted slightly different regions of the same locus were pooled for data analysis.

Sample-specific summaries:
Sample: L/L 2011-04
Specimen ID: 11062801
R/O Acute Promyelocytic Leukemia

Assays used in testing this sample:

Assay Target	Vendor*	Product Number
PML/RARA fusion	Abbott	05J70-001, 05J66-001
RARA breakapart	Abbott	05J67-001

*No endorsement of these vendors or products should be implied.

All eight participating labs reported results for a PML/RARA fusion test and two laboratories also reported results for a RARA breakapart test. All labs reported increased copy number for both PML and RARA in an average of >99% of scored cells. PML copy number ranged from 2 (in a small number of cells) to 8 and RARA copy number ranged from 2 (in a small number of cells) to 6. Similar RARA copy numbers were observed using the PML/RARA fusion and the RARA breakapart tests. However, only one lab detected a PML/RARA fusion and that was found in only 8 of 300 scored cells. Overall, the reported results are not consistent with a diagnosis of APL.

Sample: L/L 2011-05
Specimen ID: 11062802
R/O Burkitt Lymphoma

Assays used in testing this sample:

Assay Target	Vendor*	Product Number
MYC breakapart	Metasystems	D-5010-100RG
MYC breakapart	Abbott	05J91-001
IGH/MYC/CEP8 fusion (IGH/MYC)	Abbott	05J75-001
BCL6 breakapart	Abbott	05J68-001
IGH/BCL2 fusion	Abbott	05J71-001

*No endorsement of these vendors or products should be implied.

Five labs reported results for the MYC breakapart test. All of these labs reported that MYC was rearranged in approximately 95% of scored cells and that MYC copy number was normal in most of these cells. Several labs reported a smaller second population of cells in which MYC was both rearranged and increased in copy number, with up to six copies per cell. A small number of cells normal for MYC were reported by several labs. In contrast, there were no IGH/MYC rearrangements reported by the six labs that performed the test. Copy number of IGH, MYC, and CEP8 was increased in approximately 6% of scored cells, to a maximum of 4 copies. The laboratory reporting BCL6 and IGH/BCL2 results detected increased BCL6 copy number in approximately 12% of cells and increased IGH and BCL2 copy number in approximately 6% of cells but no BCL2 or IGH/BCL2 rearrangements. The rearrangement partner for MYC is not IGH but may be the kappa or lambda light chain gene, which would be consistent with Burkitt's Lymphoma.

Sample: L/L 2011-06
Specimen ID: 11062803
R/O Mantle Cell Lymphoma

Assays used in testing this sample:

Assay Target	Vendor*	Product Number
IGH/CCND1 fusion	Metasystems	D-5021-100 RG
IGH/CCND1 XT fusion	Abbott	05J72-001
IGH/CCND1 fusion	Abbott	05J69-001
CCND1 breakapart	Abbott	05J96-001

*No endorsement of these vendors or products should be implied.

All participating labs used an IGH/CCND1 fusion test for this sample and one lab also used a CCND1 breakapart test. All labs reported normal copy number and lack of rearrangement for both tests in an average of >99% of scored cells. A small number of cells with altered copy number of IGH and CCND1 was observed by some labs reporting the IGH/CCND1 fusion test. However, no rearrangements were reported for either test, and the results are not consistent with a diagnosis of Mantle Cell Lymphoma.

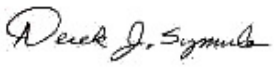
Comments on ISCN nomenclature:
There should be a slant line ("/"), not a comma, between descriptions of cell populations detected for a given test.

Other Comments
It is important to fill out the score sheets completely, including any probe or assay used in testing the sample. Assays that are used primarily to detect rearrangements may also reveal copy number changes; please report both rearrangements and copy number changes in the appropriate locations on the score sheets. Report copy number changes for individual probes, including 5' and 3' probes of a breakapart test, in the copy number section of the score sheets. Please use current catalog numbers for Abbott products to facilitate comparisons among labs.

Detailed results
Each of the enclosed sample tables gives detailed results for each assay and each sample. Each table lists the assays that were used for testing a sample and gives the average number of cells having various copy numbers or rearrangements for each assay. The copy numbers of the different probes in each fusion or breakapart (ba) assay are listed separately in these tables unless there were no reported differences for the probes in a given assay. The "# labs concordant/ #labs testing" column gives the fraction of labs that reported satisfactory results for that assay in that sample.

The "Your Score" column on the enclosed sample tables reads "concordant" if the result reported by your laboratory is consistent with that reported by the other laboratories, "not evaluable" if fewer than three labs reported results for that assay, and "not scored" if your laboratory did not perform that test on that sample. In addition, each lab received a "sample score" for each sample, based on the fraction of evaluable assays performed by that laboratory that were scored as concordant, and an overall proficiency test result of "satisfactory" or "unsatisfactory" for the event. Please keep in mind that, while this was an educational PT, laboratories should review the results as required by New York State Lab Practice Standard PT S9.

If you have questions or comments, please contact me at 518-474-6796 or genetics.health.state.ny.us.



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