Molecular and Cellular Tumor Marker Proficiency Test Event
MCTM 10-2011
Summary of results[1]

December 28, 2011

Dear Laboratory Director,

Below is a summary and discussion of the New York State Molecular and Cellular Tumor Markers proficiency test event MCTM 10-2011 from October 25, 2011.

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 37 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. Please note that in a change from previous summary tables, only the all method consensus is shown in the table. Any discrepancy between methods is mentioned in the comments and discussed below.

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

[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

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 '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 2011-07 (Table 1):
B-cell tests: For IGH, 29 (PCR=27, SB=2) out of 30 laboratories (97%) reported no rearrangement. Likewise, nine (PCR=8, SB=1) out of ten laboratories (90%) that tested for IGK reported no rearrangement. Furthermore, no lab reported a translocation involving the IGH/BCL2 or IGH/CCND1 loci. Thus, the overall conclusion was that this sample did not contain cells with any immunoglobulin gene rearrangements.

T-cell tests: 20 out of 25 laboratories (80%) that tested for TRG by PCR found a rearrangement. The five labs (4 G, 1 I) that did not conclusively detect a TRG rearrangement used various primer combinations, suggesting reasons other than primer specificity for the discrepancy (Table 4). Twelve (PCR=11, SB=1) out of fourteen labs (86%) that tested for TRB reported a rearrangement (Table 5). The one lab that did not detect a TRB arrangement by PCR used LDT primers, and the one lab that reported no rearrangement by SB used the Dako probe. Together, these results suggest that this sample contained cells with T-cell receptor gamma and beta gene rearrangements.

H-ras: one lab detected the codon 12 mutation GGT>TGT (G12C) by sequencing.

The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, IDH2, RUNX1, and WT1, and no evidence for KSHV or HTLV1 sequences was found.

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 precursor T-lymphoblastic leukemia that expressed surface CD2, CD5, CD7, CD8 and CD38, the cells of which comprised approximately 37% of the total cell population.

NYS#L/L 2011-08 (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 D that target all three framework regions and DH regions 1-6, but not with the tube E that targets only the DH 7 region. In contrast, labs that used the IVS (not Biomed-2) primers only detected a rearrangement with the FR2 and for the most part the FR3 primer mixes, but not the FR1 primer mix (Table 2). Rearrangements in IGK were detected with both Biomed-2 tubes A and B (Table 3), as well as LDT primers (one lab). The results from Southern blot (IGH=2, IGK=1) were concordant with those from PCR. No lab reported a translocation involving the IGH/BCL2 or IGH/CCND1 loci. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

IGHV mutation: Nine labs (100%) reported IGHV hypermutation, and assigned it to the IGHV3-7 family with an average mutation rate of 7.17% (range 6.73-7.5%).

T-cell tests: 19 out of 26 laboratories (73%) that tested for TRG by PCR found no rearrangement; of the seven labs that reported a rearrangement three used Biomed-2, three used LDT, and one used IVS (not Biomed-2) primers. In contrast, all fourteen labs (100%) that tested for TRB reported no rearrangement (PCR=12, SB=2). These results suggest that this sample did not contain cells with a TRB gene rearrangement, whereas at 73% the majority result for TRG is just short of the 80% required for a consensus. However, there was no clear pattern with regard to primers used among the seven labs (27%) that did detect a rearrangement.

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

P53: Two labs detected the M237I mutation in exon 7 (c.711G>A, g.13348G>A) by sequencing. Please note, according to the IARC TP53 database (http://www-p53.iarc.fr/MutationValidation.asp?Mutant=M237I) this mutation is at genomic position g.13348, not g.14038 as indicated by one lab.

The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, IDH2, RUNX1, and WT1, and no evidence for KSHV or HTLV1 sequences was found.

In aggregate, these results indicate that the sample contained a clonal B-cell population with hypermutation in the IGHV region and presence of EBV DNA. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of a B-cell clone positive for CD19, CD20, CD22, CD23, CD38, CD45 and HLA-DR, the cells of which comprised approximately 30% of the total cell population.

NYS#L/L 2011-09 (Table 1):
B-cell tests: For IGH, 26 out of 30 laboratories (87%) reported no rearrangement by PCR. Of the four labs that reported a rearrangement two used Southern blot, and two used the Biomed-2 tube D that detects rearrangements in the DH regions 1-6 (Table 2). Thus, it appears that the IGH rearrangement in this sample occurred in the DH 1-6 regions that are not targeted by the FR1-3 primers used by the vast majority of labs. 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 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 a somewhat rare rearrangement in the DH region of the IGH gene.

T-cell tests: All 26 laboratories that tested for TRG by PCR found no rearrangement. Likewise, all fourteen labs that tested for TRB reported no rearrangement (SB=2, PCR=12). These results suggest that this sample did not contain cells with T-cell receptor gene rearrangements.

EBV: All four labs 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, IDH2, RUNX1, and WT1, and no evidence for KSHV or HTLV1 sequences was found.

In aggregate, these results indicate that the sample contained a clonal B-cell population. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of a B-cell clone positive for CD19, CD2, CD38 and HLA-DR, but not surface expression of kappa or lambda, the cells of which comprised approximately 11% of the total cell population.

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, Tables 6-9 show 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/L7, 8, and 9 ranged from a minimum of 0.3, 1.0, and 0.5 µg/ml to a maximum of 375, 230, and 246 µg/ml, respectively, corresponding to a 230- to 1250-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L7, 8 and 9 also ranged broadly from 1.2, 0.8, and 0.8 µg/ml to 287.3, 228.5, and 115 µg/ml, respectively, corresponding to a 143- to 239-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. 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 will be a change to TWO Molecular and Cellular Tumor Marker PT mail-outs in 2012 with the following dates:

Mail-out date	**Due Date**
March 20, 2012	April 18, 2012
October 23, 2012	November 21, 2012

Sincerely,

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

New York State Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 10-2011
Table 1: Summary of results

Assay / Sample	L/L 2011-07					L/L 2011-08					L/L 2011-09				
	R	G	ind	N	Cons#	R	G	ind	N	Cons#	R	G	ind	N	Cons#
IGH	1	29			G	30				R	4	26			G
IGK	1	9			G	10				R	10				R
TRB	12	2			R		14			G		14			G
TRG	20	4	1		R	7	19			I		26			G
IGHV (IgVH)				8	N	9				R				8	N
IGH/BCL2 t(14;18): MBR		11			G		11			G		11			G
IGH/BCL2 t(14;18): mcr		8			G		8			G		8			G
IGH/BCL2 t(14;18): MBR 3'		3			G		3			G		3			G
IGH/CCND1 t(11;14)		4			G		4			G		4			G
JAK 2 V617F		30			G		30			G		30			G
JAK 2 Exon 12		10			G		10			G		10			G
MPL W515		10			G		10			G		10			G
MPL S505		7			G		7			G		7			G
FLT 3 ITD		6			G		6			G		6			G
FLT 3 D835		5			G		5			G		5			G
NPM1		11			G		11			G		11			G
CEBPA		5			G		5			G		5			G
IDH1		2			I		2			I		2			I
c-kit		8			G		8			G		8			G
BCR/ABL1 t(9;22): p210		27	1		G		27	1		G		27	1		G
BCR/ABL1 t(9;22): p190		27			G		27			G		27			G
BCR/ABL1 t(9;22): p210/190		10			G		9			G		9			G
Abl kinase domain mutation		2	2		I		2	2		I		2	2		I
PML/RARA t(15;17): Long		13			G		13			G		13			G
PML/RARA t(15;17): Short		13			G		12			G		12			G
PML/RARA t(15;17): Variable		4			G		4			G		4			G
MYC t(8;14)					-					-					-
AML1/ETO t(8;21)		5			G		5			G		5			G
NPM/ALK t(2;5)					-					-					-
ETV6/RUNX1 t(12;21) (Tel-AML1)		2			I		2			I		2			I
CBFB/MYH11 INV(16)		2			I		2			I		2			I
E2A-PBX1 t(1;19)		1			I		1			I		1			I
MLL (11q23)/AF4 t(4;11)		2			I		2			I		2			I
P53		2			I	2				I		2			I
K-Ras		2			I		2			I		2			I
N-Ras	1				I		1			I		1			I
H-Ras		1			I		1			I		1			I
EBV		4			G	4				R	4				R
Interpretation:	Clonal T-cell population with TRB and TRG rearrangements; approx. 37% of total cell population					Clonal B-cell population wit IGH and IKG rearrangement and IGHV hypermutation; approx. 30% of total cell population					Clonal B-cell populaiton with IGK rearrangement and rare rearrangement in the DH region of the IGH gene; approx. 11% of total cell population				
Comments											IGH: 2 SB and 2 PCR Biomed-2 tube D results are positive.				

R: rearranged/mutated/translocated or otherwise aberrant; G: germline/wildtype or otherwise no evidence of abnormality; I: indeterminate, a clear interpretation is not possible
N: no clonal band detected
Cons#: Consensus based on ≥80% concordance; I if no consensus or <3 results

New York State Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 10-2011
Tables 2-5: Individual primer results

Table 2: Summary for IGH primer mixes

Reagent Source	Mix	L/L 2011-07		CONSENSUS	L/L 2011-08		CONSENSUS	L/L 2011-09		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A		9	**G**	9		**R**		9	**G**
	B		10	**G**	10		**R**		10	**G**
	C		9	**G**	8		**R**		9	**G**
	D		2	**I**	2		**I**	2	1	**I**
	E		3	**G**		3	**G**		3	**G**
IVS	FR 1		4	**G**		5	**G**		5	**G**
	FR 2		6	**G**	7		**R**		6	**G**
	FR 3		7	**G**	5	2	**I**		7	**G**
LDT	FR 1		2	**I**	1	1	**I**		2	**I**
	FR 2	1	7	**G**	8		**R**		8	**G**
	FR 3	1	11	**G**	9	2	**R**		11	**G**

Table 3: Summary for IGK primer mixes

Reagent Source	Mix	L/L 2011-07		CONSENSUS	L/L 2011-08		CONSENSUS	L/L 2011-09		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A	1	7	**G**	9		**R**	9		**R**
	B	1	7	**G**	9		**R**	9		**R**

Table 4: Summary for TRG primer mixes

Primer Source	Mix	L/L 2011-07		CONSENSUS	L/L 2011-08		CONSENSUS	L/L 2011-09		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A	11	1	**R**	1	9	**G**		12	**G**
	B	9	2	**R**	2	10	**G**		11	**G**
LDT	Vγ1-8	2	2	**I**	1	4	**G**		5	**G**
	Vγ9	1	2	**I**		4	**G**		4	**G**
	Vγ10		4	**G**		4	**G**		4	**G**
	Vγ11		3	**G**		3	**G**		3	**G**
LDT/IVS	Mix 1	4		**R**	3	2	**I**		5	**G**
(not Biomed-2)	Mix 2	1	4	**G**	1	4	**G**		5	**G**

Table 5: Summary for TRB primer mixes

Primer Source	Mix	L/L 2011-07		CONSENSUS	L/L 2011-08		CONSENSUS	L/L 2011-09		CONSENSUS
		R	G		R	G		R	G	
Biomed-2	A	7	1	**R**		8	**G**		8	**G**
	B	8		**R**		8	**G**		8	**G**
	C	5	2	**I**		7	**G**		7	**G**

New York State Molecular and Cellular Tumor Marker Proficiency Test Event MCTM 10-2011
Tables 6-9: Summaries of methods

Table 6

	IGH	IGK	TRB	TRG	IGHV	IGH/BCL2	IGH/CCND1
Total	30	10	14	27	8	11	4
SB	2	1	2				
PCR	28	9	12	27	1	11	4
Seq					4		
PCR Seq					3		
Lab developed	11	2	4	14	6	6	3
IVS (Biomed-2)	10	8	10	12		4	
IVS (not Biomed-2)	7			1	3	1	
Lab developed and IVS (Biomed-2)	1						
Qualitative						10	3
Quantitative						1	1

Table 7

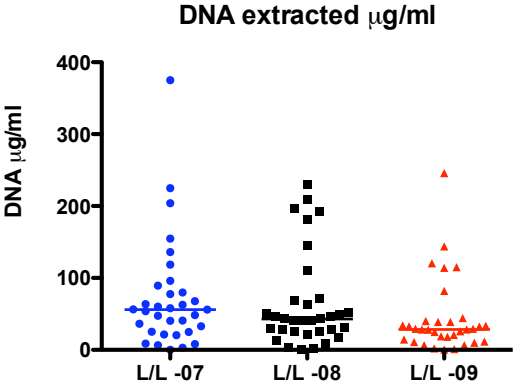
	JAK2 V617F	JAK2 Exon 12	MPL	FLT3 ITD	FLT3 D835	NPM1	CEBPA	IDH1	c-kit
Total	30	10	10	7	6	11	5	2	8
PCR	28	5	3	7	5	10	2	1	3
RT-PCR					1				
Seq	1	4	6			1	3	1	4
PCR Seq									1
RT-PCR Seq	1	1	1						
Lab developed	22	10	10	6	5	11	5	2	7
Ipsogen (Qiagen)	8								
Seegene				1	1				
Qualitative	20								
Quantitative	9								

Table 8

	BCR/ABL1	Abl kinase	PML/RARA	MYC	AML1/ETO	NPM/ALK	ETV6/RUNX1	CBFB/MYH11	E2A-PBX	MLL/AF4
Total	32	8	13		5		2	2	1	2
PCR										
RT-PCR	32	1	13		5		2	2	1	2
Seq		5								
PCR Seq										
RT-PCR Seq		2								
Lab developed	19	8	12		5		2	2	1	2
Ipsogen (Qiagen)	8		1							
Roche	3									
Cepheid	1									
Asuragen	1									
Qualitative	6		5		3		1	1	1	2
Quantitative	26		8		2		1	1		
IS Normalized	5									

Table 9

	P53	Ras	EBV	Other 1	Other 2	Other 3	Other 4
Total	2	2	4	6	4	1	1
PCR		1	4	4	2		
Seq	1			1	1		
PCR Seq	1	1		1	1	1	1
Lab developed	2	2	4	6	4	1	1



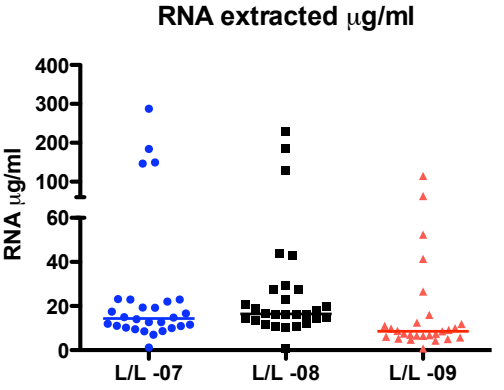


Figure 1. NYS MCTM PT 10-11 DNA and RNA yields. The yields were converted to ug DNA and RNA per 1 ml blood.

	L/L 2011-07	L/L 2011-08	L/L 2011-09		L/L 2011-07	L/L 2011-08	L/L 2011-09
	DNA	DNA	DNA		RNA	RNA	RNA
Median	56.0	43.0	28.5	Median	14.4	16.4	8.6
Min	0.3	1.0	0.5	Min	1.2	0.8	0.8
Max	375.0	230.0	246.0	Max	287.3	228.5	115.0

New York State Oncology - Molecular and Cellular Tumor Markers
Proficiency Test - October, 2011
Participant Summary - FISH Testing
January 17, 2012

Below is a summary of interphase FISH results for the October, 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 rule out a clinical diagnosis in three proficiency test samples using interphase FISH. 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 2011-07
Specimen ID: 111025-01
R/O Chronic Lymphocytic Leukemia (CLL)

Assays used in testing L/L 2011-07:

Assay Target	Vendor*	Product Number
P53	Abbott Molecular	04N02-020, 05J52-011, 05J83-001
P53	Metasystems	D-5017-100RG
13q14.3	Abbott Molecular	04N02-020, 05J15-011, 05J83-001, 05J86-011, 05J81-011
13q14.3	Metasystems	D-5006-100RG
ATM	Abbott Molecular	04N02-020, 05J64-011, 05J83-001
ATM	Metasystems	D-5011-100RG
12cen	Abbott Molecular	04N02-020, 07J20-012, 05J83-001, 06J37-022
12cen	Metasystems	D-0812-050F1
IGH/CCND1 dual fusion	Abbott Molecular	05J69-001, 05J72-001
IGH/CCND1 dual fusion	Metasystems	D-5021-100RG
17cen	Abbott Molecular	04N02-020, 05J83-001
13q34	Abbott Molecular	04N02-020, 05J83-001
MYB	Abbott Molecular	07J86-011

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

All participating laboratories tested this sample for aberrations in P53, ATM, the 13q14.3 region, and chromosome 12, loci commonly deleted in CLL. All laboratories reported increased copy number for all four of these loci in the majority of cells and normal or decreased copy number in a few cells. The laboratories that included an IGH/CCND1 assay reported increased copy number for the majority of cells analyzed and no rearrangements. Similarly, the three laboratories that assayed MYB found increased copy number in the majority of cells. Overall, participating laboratories reported increased copy number in the majority of cells analyzed for all loci tested, which is inconsistent with a diagnosis of CLL.

_____

Sample: L/L 2011-08
Specimen ID: 111025-02
R/O Myelodysplastic Syndrome (MDS)

Assays used in testing L/L 2011-08:

Assay Target	Vendor*	Product Number
5q31-32	Abbott Molecular	05J60-001, 05J76-001
5q31-32	Metasystems	D-5024-100RG
5p15	Abbott Molecular	05J60-001, 05J76-001
5p15	Metasystems	D-5024-100RG
7q31	Abbott Molecular	05J61-001
7q31	Metasystems	D-5025-100-RG
20q12	Abbott Molecular	05J47-011
20q12	Metasystems	D-5020-100-RG
MLL ba	Abbott Molecular	05J90-001
MLL ba	Metasystems	D-5013-100-RG
AML1/ETO df	Metasystems	D-0826-050-OG
7cen	Abbott Molecular	05J61-001
8cen	Abbott Molecular	07J20-008, 06J54-018, 06J37-018

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

All participating laboratories reported results for chromosome regions 5q31-32, 7q31, and 20q12 and consistently reported that a majority of cells had normal copy number for all of these loci. Decreased copy number of any one of these loci, as would be expected for MDS, was detected in a maximum of 4% of analyzed cells. Seven of these laboratories also reported results for chromosome 8 copy number and reported trisomy 8 in up to 15% of analyzed cells. Several laboratories reported results for an MLL breakapart assay that showed no evidence of rearrangement and normal copy number. While labs did not report normal cutoff values, the low frequency of deletion of 5q31-32, 7q31, 20q12 likely would be inconsistent with a diagnosis of MDS. However, the presence of trisomy 8 at these frequencies might preclude ruling out a diagnosis of MDS, depending on the normal cutoff values for this test used in each lab.

Sample: L/L 2011-09
Specimen ID: 111025-03
R/O Acute Promyelocytic Leukemia

Assays used in testing L/L 2011-09:

Assay Target	Vendor*	Product Number
PML/RARA df	Abbott Molecular	05J70-001, 05J66-001
PML/RARA df	Metasystems	D-5023-100-RG
RARA ba	Abbott Molecular	05J67-001

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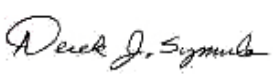
All participating laboratories reported results for a PML/RARA fusion assay and two of these reported results for a RARA breakapart assay. All laboratories were consistent in finding that the majority of cells had normal copy number for these loci, with small numbers of cells having increased or decreased copy number of one or both. However, the maximum number of signals for PML and RARA varied widely, up to a maximum of 20. Only one laboratory reported PML/RARA fusion and this rearrangement was found in only 13 of 300 cells analyzed. The reported results are not consistent with a diagnosis of APL.

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 together in these tables unless the probes in a given assay differed. 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 tested" if your laboratory did not perform that test on that sample.

In addition, each lab received a separate Score Sheet "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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