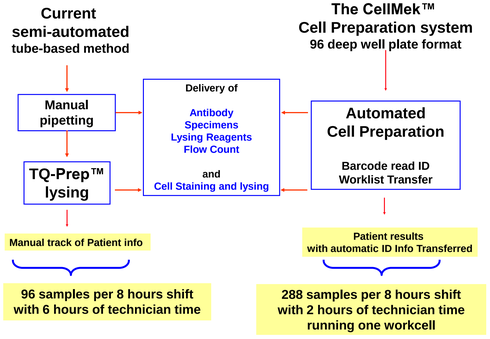
An Automated Cell Preparation Method For FlowCARE™ Pan-Leukocyte Gating (PLG) CD4 Assay in a 96-Well Plate Offers Increased Throughput and Ease of Use

Jin Zhang, Valentin Quesada, Juan De Castro, Jennifer Zawislak, Jorge Acevedo, Ed Jachimowicz, and Liliana Tejidor
Beckman Coulter, Inc., Cellular Analysis Business Group, Miami, FL

ABSTRACT

Background. The measurement of CD4+ cells in HIV-infected patients has evolved into a routine and high volume test in flow cytometry. CD4+ cell enumeration is measured with differing combinations of monoclonal antibodies, balancing performance with cost. A good balance is the PLG CD4 assay, using two monoclonal antibodies: anti-CD45 for resolving leukocytes and anti-CD4 for enumerating CD4+ cells. The current PLG assay is semi-automated and allows for a maximum of 32 blood samples in tubes to be processed in a batch.
Methods. We have modified the FlowCARE™ PLG CD4 assay so that the addition of blood, antibodies, lysing, quenching, fixation reagents, and Flow-Count™ fluorospheres is performed in 96-well plates by an automated cell preparation system CellMek™. The plate is then transferred to a flow cytometer equipped with a multi-plate reader for enumeration of CD4+ cells. For identification purposes, each individual sample is tracked throughout by bar code. There is an approximately 30% reduction in time between the semi-automated and automated systems.
Results. Performance testing of 24 non-clinical and 70 HIV samples demonstrated a significant correlation between the FlowCARE PLG CD4 assay prepared in 96-well plates on the CellMek and the semi-automated tube method ($r^2 = 0.99$). Aging studies with 9 non-clinical and 15 HIV specimens (up to 5 days post blood draw) showed no significant change in the absolute CD4+ counts and percent of CD4+ cells ($p = 0.41/0.99$). This application is undergoing clinical evaluation.
Conclusions. The results indicate that the PLG assay using automated sample preparation in a 96-well plate system gives comparable performance to the semi-automated tube method. The ability to process larger batches of clinical samples in a shorter period of time allows for reduced costs, higher throughput, and more efficient use of laboratory staff.

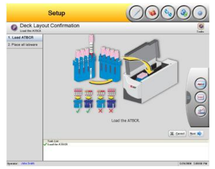
SYSTEM OVERVIEW

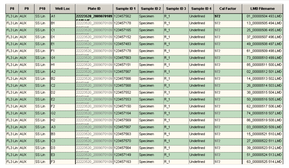


Key Features of the CellMek Cell Preparation System

- Simple user interface and requires minimal skill to operate with guided workflow.
- Processes primary specimens, no need for daughter tubes.
- Prepares 96 specimens (and controls) in <1.5 hours in one batch requiring <15 minutes technician hands on time on CellMek.
- System configurable to create work cell capable of processing approximately 6 plates (~ 576 samples) in an 8 hour shift with two workcells.
- Reagent and process management includes verification of on-board reagents prior to plate processing.
- Automated specimen and plate ID transfer through barcode reading and worklist transfer from CellMek to cytometer (via shared network).
- Results in spreadsheet, viewed in a workstation, and available to interface with LIS.

Menu-driven software to set up processing

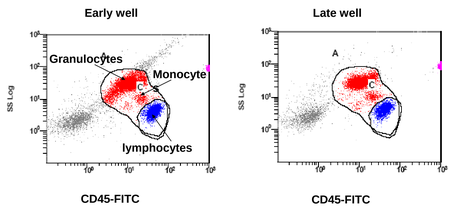




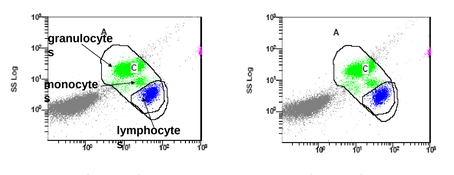
RESULTS

Representative Assay Results

CellMek Processed Blood Samples



Current TQ-Prep Processed Blood

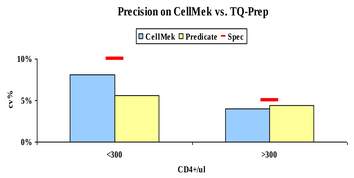


Repeatability

Methods	Donors	Replicates	
CellMek	34 donors 0-5 day aged Run over 4 days	10	Randomized on 96 well plate
Predicate	18 donors 0-5 day aged Run over 4 days	10	Manual preparation

	CellMek		Predicate	
CD4+/ul Range	# of Donor	pooled cv%	Donor	pooled cv%
<100	5	8.9%	2	5.6%
100-199	6	6.4%	1	2.9%
200-350	6	7.4%	7	5.5%
351-500	6	3.7%	1	3.3%
>500	7	3.9%	4	4.0%
<300	15	8.1%	7	5.6%
>300	15	4.0%	8	4.4%

CellMek and predicate used different set of donors

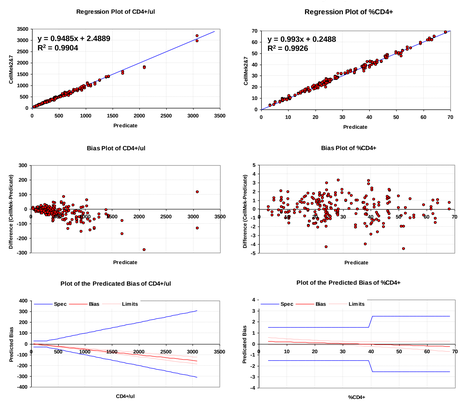


Accuracy

Compare the accuracy of the test method on CellMek to the predicate method (TQ-Prep/MCL) using multiple donors over 96 well plate following CLSI EP9-A FDA guidelines. Results showed that the CellMek Cell Preparation System gives equivalent CD4 results as the semi-automated method.
Sample preparation:

Methods	Donors	Replicates	
CellMek	24 nonclinical/70 abnormal donors 0-5 day aged Repeated on 2 Cellmek Units	2	Randomized on 96 well plate
Predicate	24 nonclinical/70 abnormal donors 0-5 day aged	2	Manual preparation

CD4+ Absolute Count



%CD4+ of lymphocytes

Flow Count Stability in Prepared Samples

A 96 well plate with a mixture of normal and abnormal specimens will take about 3-4 hours to complete the analysis on cytometer. Prepared samples (with the addition of Flow Count Fluorospheres) have shown stability for at least 6 hours after preparation. 15 abnormal and 9 nonclinical specimens were used and each donor was duplicated and randomized.
Samples preparation:

Flow Count Lots Tested	Specimens	Replicates	Time points for analysis
3	15 abnormals 9 nonclinicals per lot	One plate with duplicates per specimen	Fresh or 6 hours

Paired t-test was performed to compare the data from the 6_hours run to time zero run using the bias specs. P > 0.05 indicates samples are stable when analyzed within 6 hours after preparation on the Cellmek.

	Flow Count Lot #1_7548046			
	CD4+/ul		%CD4+	
Parameter	Fresh	6 hrs	Fresh	6 hrs
Spec %	10%			
spec	53.67		1.5	
Mean	536.71	537.23	29.29	29.42
SD	312.66	311.70	12.86	12.92
Diff		0.52		0.13
Correl		0.9959		0.9963
SE		28.23		1.11
p-value		1.0000		1.0000

	Flow Count Lot #2_7548055			
	CD4+/ul		%CD4+	
Parameter	Fresh	6 hrs	Fresh	6 hrs
Spec %	10%			
spec	50.88		1.5	
Mean	508.78	503.80	32.04	32.09
SD	410.40	405.10	17.96	17.89
Diff		-4.98		0.05
Correl		0.9976		0.9984
SE		28.61		1.01
p-value		1.0000		1.0000

	Flow Count Lot #3_7548053			
	CD4+/ul		%CD4+	
Parameter	Fresh	6 hrs	Fresh	6 hrs
Spec %	10%			
spec	31.91		1.5	
Mean	319.11	319.10	26.72	27.32
SD	349.68	349.32	21.20	20.86
Diff		-0.02		0.60
Correl		0.9976		0.9893
SE		24.35		3.10
p-value		1.0000		0.9886

Specimen Stability

To show specimen stability is 5 days post venipuncture using specimens processed on CellMek Cell Preparation System. Each sample and duplicates were randomized on the plate.

Specimens	Replicates	Plates	Time Points for analysis
15 abnormals 9 nonclinical specimens	duplicates per specimen	One plate Per day point	Day 0 Day 5 Day7

Paired t test was performed to compare the CD4+/ul and %CD4+ of Day 5 and 7 to that of Day Zero by using the bias spec. P > 0.05 indicates the system will support specimen stability of up to five (5) days between venipuncture and testing when kept at room temperature (20-25ºC) for CD4+ cell enumeration.

	CD4+/ul			CD4+/ul		
Parameter	Day 0	Day 5	Day 7	Day 0	Day 5	Day 7
Spec %	10%					
spec	57.9104			1.5		
Mean	579.104	518.604	503.229	28.01	28.87	28.06
SD	574.431	510.703	494.055	18.29	18.94	18.50
Diff		-60.5	-75.875		0.86	0.05
Correl		0.99735	0.99621		0.9970	0.9967
SE		74.9545	92.8049		1.59	1.50
p-value		0.40593	0.09316		0.9963	1.0000

Prepared Sample Stability

To show the stability of the prepared samples (without the addition of Flow Count Fluorospheres) is 24 hours when stored in a refrigerator following specimen preparation. 15 abnormal and 9 nonclinical specimens were used and each donor was duplicated and randomized.
Sample preparation:

Plate	Fresh	24 hours	48 hours
Prep on CellMek	PLG with Flow Count	PLG w/o Flow Count	PLG w/o Flow Count
Store in the refrigerator		24 hours	48 hours
Bring to RT		Yes	Yes
Load Flow Count on CellMek		Run Beads Only Method	Run Beads Only Method
Analysis on the Cytometer	Immediately after Prep	After loading the Beads	After loading the Beads

Paired t test was performed to compare the CD4+/ul and %CD4+ of 24 hrs and 48 hrs plates to the 0 hr plate using the bias spec. P > 0.05 indicates that the system will support the lysed specimen stability of up to 24 hours if stored at 2 – 8ºC for CD4+ cell enumeration.

	CD4+/ul			%CD4+		
Parameter	Fresh	24 hrs	48 hrs	Fresh	24 hrs	48 hrs
Spec %	10%					
spec	52.82			1.5		
Mean	528.19	526.35	534.89	29.06	28.85	28.86
SD	317.41	317.91	328.74	12.89	13.26	13.31
Diff		-1.84	6.70		-0.21	-0.19
Correl		0.9913	0.9911		0.9964	0.9967
SE		41.92	44.57		1.17	1.14
p-value		1.0000	1.0000		1.0000	1.0000

CONCLUSIONS

- The CellMek Cell Preparation System provides an automated CD4 counting system customized for the South African National Health System.
- Features include:
  - A high-throughput plate-based solution
  - Extendable work-cell configuration (up to 2 CellMek systems and 4 cytometers)
  - Use of existing PLG CD4 reagents
  - Extension of reagent stability up to 5 days
- The Cellmek Cell Preparation System is able to deliver accurate and precise results equivalent to current tube-based methods using larger batch sizes in a shorter period of time.

ACKNOWLEDGEMENTS

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