Streck Cell Preservative™ preserves bone marrow specimens

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Abstract

Bone marrow specimens were preserved with Streck Cell Preservative to determine the efficacy of the reagent for the stabilization of immune epitopes on the white blood cells for flow cytometry analysis. Stabilization of bone marrow samples allows the laboratory to store samples for further analysis, to batch similar samples, and to reduce weekend staffing.

Leukemias and lymphomas are cancers of the blood and lymphatic systems. Flow cytometry analysis of the white blood cells from bone marrow aspirates is used to diagnose these conditions. Bone marrow is the soft tissue that resides within some of the larger bones and its function is to produce blood cells. Bone marrow testing is performed because it is more likely that abnormal white blood cells will be found in the bone marrow than in the bloodstream. The proportion of white blood cells that are abnormal is also important for diagnosis.

Introduction

A bone marrow specimen is a difficult sample to retrieve and requires a skilled healthcare professional to obtain the specimen. These samples must be analyzed by the flow cytometry facility immediately, requiring technologists to be on-call to process samples over the weekend. Flow cytometry laboratories have a general panel of Cluster of Differentiation (CD) markers indicative of leukemia or lymphoma used for the flow cytometry assay. After reviewing these results, the physician may require a more specific panel of CD-markers in order to properly diagnose the disease.

Streck Cell Preservative is a cellular preservative and transport reagent that stabilizes the cell-surface antigens on white blood cells for immunophenotyping by flow cytometry. Additionally, Streck Cell Preservative is easy to use and only requires a 1:1 (v/v) sample to reagent dilution. A bone marrow aspirate can be preserved in Streck Cell Preservative until the technologist has adequate time to process the sample for flow cytometry. The data presented in this technical communication illustrate the utility of Streck Cell Preservative to stabilize the immune epitopes on the white blood cells in bone marrow specimens.

Materials and Methods

Sample Preparation for Flow Cytometry

Bone marrow aspirates were obtained, placed into 3 mL EDTA blood collection tubes and mixed by inversion 5-10 times. A 1.0 mL aliquot of bone marrow was added to a vial containing 1.0 mL of Streck Cell Preservative. Streck Cell Preservative treated samples were then stored at 2 ºC to 8 ºC.

A 2 mL aliquot of Streck Cell Preservative treated bone marrow was placed into a 15 mL conical tube with 5 mL of Hanks solution and mixed gently by inversion. The sample was then centrifuged for five minutes at 2000 rpm and the supernatant was aspirated from the cell pellet. The cell pellet was then mixed with 13 mL of ammonium chloride, mixed gently by inversion and placed in the dark for five minutes to lyse the red blood cells. The cell suspension was then centrifuged for five minutes at 2000 rpm and the supernatant was aspirated from the cell pellet. Thirteen mL of Hanks solution was then added and the sample was mixed to stop the lysing action. The cell mixture was centrifuged for 5 minutes at 2000 rpm. The supernatant was aspirated from the cell pellet and the cells were vortexed with 1 mL of 2% newborn bovine serum (NBS). A viability count was performed in order to make a cell suspension of $2x10^7$ cells/mL and the amount of NBS was adjusted accordingly. This protocol is outlined in Figure 1.

Slides were prepared for Wright-Giemsa staining throughout the cell manipulation process to monitor the consistency of leukemic cells compared to the original sample.

Flow Cytometry Analysis

Flow cytometric analysis was performed on a FACSCalibur™ instrument (BD Biosciences, San Jose, CA). Briefly, 50 µL of the bone marrow cell suspension was incubated with 20 µL of FITC, PE and PerCP-Cy5.5 labeled monoclonal antibodies and with 5 µL of APC

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labeled monoclonal antibodies. The antibodies and fluorescent conjugates tested are listed in Table 1. Samples were then vortexed at low speed for three seconds and placed at 2 °C to 8 °C in the dark for 20 minutes. Three mL of PBS with 0.1% azide and albumin were added to each tube. This was followed by centrifugation at 800 rpm for five minutes. All but approximately 50 µL of supernatant was aspirated from the cell pellet and the sample vortexed for three seconds at low speed. One mL of 1% formaldehyde was added to the cell mixture and vortexed briefly. Samples were placed at 2 °C to 8 °C prior to analysis by flow cytometry.

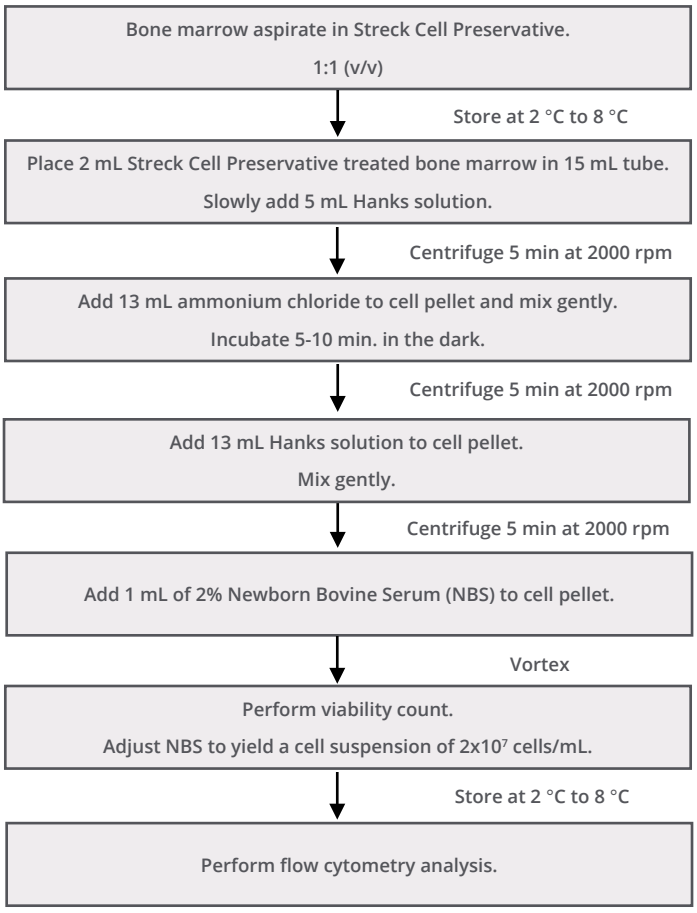


Figure 1. Streck Cell Preservative-treated bone marrow sample processing protocol.

Flow Cytometry Instrument Set Up

The FACSCalibur flow cytometer was calibrated daily with CaliBRITE™ beads and FACSComp software (BD Biosciences). Instrument settings used were those established by FACSComp. Samples were evaluated using CellQuest software (BD Biosciences). Percent recovery values were recorded for lymphocyte subsets.

Table 1. Leukemia and lymphoma panel of antibodies.

FITC	PE	PerCP-Cy5.5	APC
Auto	Auto	CD45	Auto
CD5	CD19	CD45	CD23
CD3	CD8	CD45	CD4
CD10	CD34	CD45	
CD25	CD22	CD45	CD11c
Kappa	CD19	CD45	CD20
Lambda	CD19	CD45	CD38
CD56	CD16	CD45	CD5
CD2	CD7	CD45	
CD15	CD14	CD45	
CD41a	CD13	CD45	CD33
HLA-DR	CD7	CD45	
MPO	CD19	CD45	CD79a

Results

Ten benign and 10 neoplastic bone marrow specimens were analyzed by flow cytometry on the day they were collected. The samples were then treated with Streck Cell Preservative and analyzed on day three. Figures 2 and 3 show the scattergrams obtained for CD19, Lambda, and CD38 from a bone marrow sample analyzed by flow cytometry on the day of receipt and at day three. The data indicates that Streck Cell Preservative stabilizes the immune epitopes of the white blood cells in bone marrow aspirates with negligible effect on the light scatter and fluorescence profiles.

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Table 2 illustrates the percent recoveries attained from a representative bone marrow sample for all markers tested. Most of the CD markers analyzed showed only slight increases or decreases in relative fluorescence intensity over the three-day period. These slight phenotypic differences are not significant enough to alter the diagnosis of the patient. Some markers, such as CD8 and CD11c, displayed larger variabilities of +20% and –15%, respectively. This variation can be attributed to slight shifts in the cell populations caused by the Streck Cell Preservative preservative. A skilled flow cytometrist may compensate for these shifts; however, the quadrant markers in this study were not moved in order to provide the most credible data.

Table 2. Percent recoveries for 27 CD-markers from a bone marrow sample analyzed on the day of receipt (untreated) and at day three (treated with Streck Cell Preservative). The percent difference between the two samples is also shown.

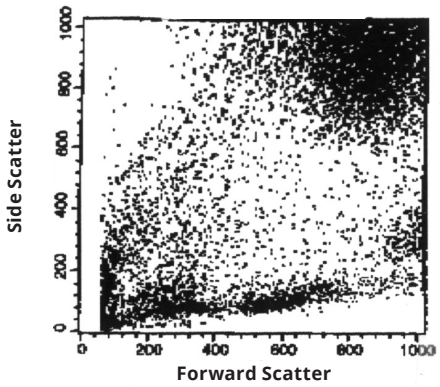
Marker	Percent Recovery Initial Day	Percent Recovery 3-Days	Percent Difference
CD2	43%	49%	6%
CD3	37%	38%	1%
CD4	23%	18%	-5%
CD5	36%	32%	-4%
CD7	37%	44%	7%
CD8	29%	49%	20%
CD10	41%	39%	-2%
CD11c	23%	8%	-15%
CD13	29%	18%	-11%
CD14	19%	26%	7%
CD15	29%	33%	4%
CD16	19%	17%	-2%
CD19	34%	39%	5%
CD20	35%	23%	-12%
CD22	43%	51%	8%
CD23	3%	0.3%	-3%
CD25	8%	8%	0%
CD33	15%	3%	-12%
CD34	22%	19%	-3%
CD38	59%	54%	-5%
CD41a	10%	4%	-6%
CD56	8%	7%	-1%
CD79a	17%	23%	5%
Kappa	26%	42%	16%
Lambda	19%	27%	8%
HLA-DR	72%	74%	2%
MPO	14%	16%	2%

Discussion

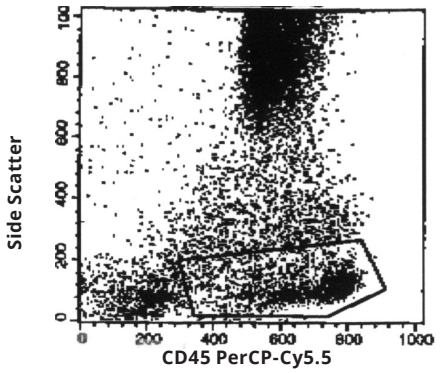
The data presented in this technical brief establishes that Streck Cell Preservative extends the stability of the white blood cell immune epitopes in bone marrow aspirates for three days. While the samples in this study were only analyzed through day three, samples may be stable for a longer timeframe. The ability to maintain bone marrow aspirates for three days or longer is a significant advance for the clinical laboratory. Implementation of Streck Cell Preservative will allow the laboratory to better manage workflow and to reduce weekend labor costs. Streck Cell Preservative also provides significant advantages for the patient by eliminating sample rejection due to transportation or processing delays and by extending the storage time, which allows the laboratory to retain the sample for additional testing. These benefits reduce the likelihood of patient redraws, which are costly for the clinical laboratory and painful for the patient.

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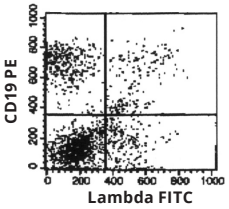
Figure 2. Untreated bone marrow aspirate analyzed by flow cytometry for CD19, CD38 and Lambda on the day of receipt.



Region	Events	%Gated	%Total	XMean	YMean	Px.Py
R1	2104	14.03	14.03	482.46	122.68	5,2

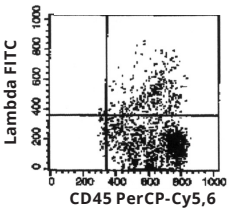


Region	Events	%Gated	%Total	XMean	YMean	Px.Py
R1	2104	14.03	14.03	482.46	122.68	5,2



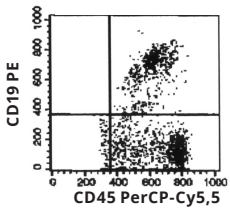
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Gated Events: 2120

Quad	Events	%Gated	%Total
UL	547	28.00	3.65
UR	185	8.79	1.23
LL	1148	54.56	7.65
LR	224	10.65	1.49



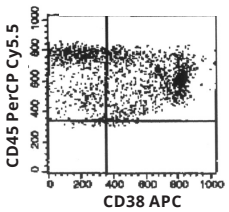
Gate :G1
Gated Events: 2120

Quad	Events	%Gated	%Total
UL	12	0.57	0.08
UR	391	18.58	2.16
LL	52	2.47	0.35
LR	1619	76.37	10.99



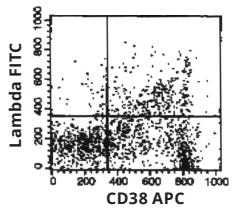
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Gated Events: 2120

Quad	Events	%Gated	%Total
UL	1	0.05	0.01
UR	717	34.08	4.78
LL	84	3.99	0.56
LR	1302	61.88	8.68



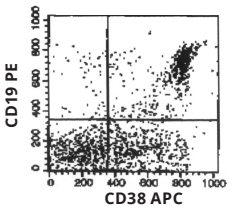
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Quad	Events	%Gated	%Total
UL	817	38.83	5.45
UR	1227	58.32	8.18
LL	31	1.47	0.21
LR	29	1.38	0.19



Gate :G1
Gated Events: 2120

Quad	Events	%Gated	%Total
UL	53	2.52	0.35
UR	360	17.11	2.40
LL	763	37.21	5.22
LR	908	43.16	8.05

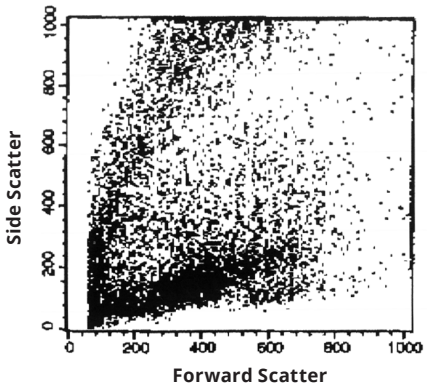


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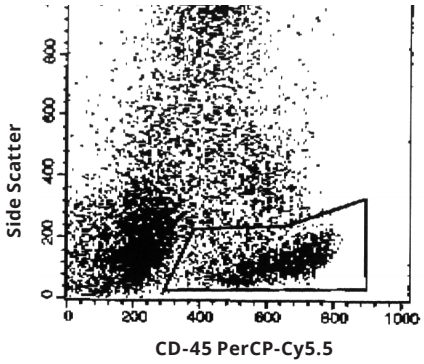
Quad	Events	%Gated	%Total
UL	57	2.71	0.38
UR	681	32.87	4.54
LL	808	38.40	5.39
LR	568	26.52	3.72

Streck Cell Preservative™ preserves bone marrow specimens

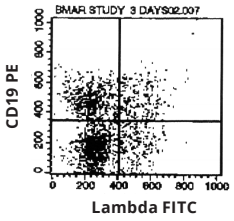
Figure 3. Streck Cell Preservative-treated bone marrow aspirate analyzed by flow cytometry for CD19, CD38 and Lambda on day three.



Region	Events	%Gated	%Total	XMean	YMean	Px.Py
R1	2120	14.13	14.13	354.94	128.49	5,2

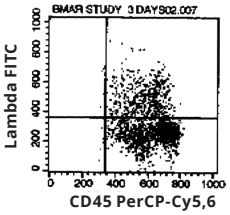


Region	Events	%Gated	%Total	XMean	YMean	Px.Py
R1	2120	14.13	14.13	354.94	128.49	5,2



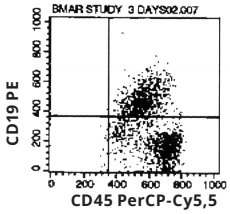
Gate :G1
Gated Events: 2120

Quad	Events	%Gated	%Total
UL	614	28.96	4.09
UR	253	11.93	1.69
LL	1019	48.07	6.79
LR	234	11.04	1.56



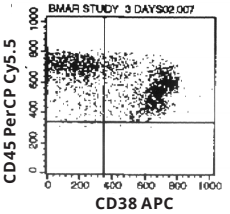
Gate :G1
Gated Events: 2120

Quad	Events	%Gated	%Total
UL	5	0.24	0.03
UR	596	28.11	3.97
LL	9	0.42	0.06
LR	1510	71.23	10.07



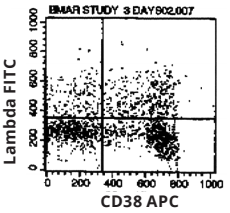
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Gated Events: 2120

Quad	Events	%Gated	%Total
UL	2	0.09	0.01
UR	817	38.54	5.45
LL	21	0.99	0.14
LR	1280	60.38	8.53



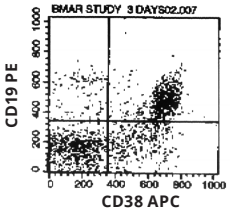
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Quad	Events	%Gated	%Total
UL	976	46.04	6.51
UR	1130	53.30	7.53
LL	8	0.38	0.05
LR	6	0.28	0.04



Gate :G1
Gated Events: 2120

Quad	Events	%Gated	%Total
UL	218	10.28	1.45
UR	418	19.72	2.79
LL	759	35.80	5.06
LR	725	34.20	4.83



Gate :G1
Gated Events: 2120

Quad	Events	%Gated	%Total
UL	128	6.04	0.85
UR	747	35.24	4.98
LL	864	40.75	5.76
LR	381	17.97	2.54

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