Cyto-Chex® Reagent Stabilizes Bone Marrow Cells and their Antigen Expression Profiles for Extended Analysis Using Flow Cytometry

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Abstract

Streck Cyto-Chex® reagent is a cell and tissue preservative used to stabilize samples for analysis by flow cytometry. Current flow cytometry protocols require analysis of bone marrow samples within 24 hours of collection. The goal of this study was to determine whether bone marrow is preserved beyond 24 hours using Cyto-Chex reagent, thus avoiding the rejection of samples delayed during shipping and eliminating the need for weekend staffing. We report that Streck Cyto-Chex reagent can preserve bone marrow samples for flow cytometric analysis for 72 hours. Bone marrow samples drawn from patients were mixed 1:1 with Cyto-Chex and evaluated by flow cytometry at 6 hours and 72 hours after isolation. Thirteen patient samples were tested for CD marker expression in standard leukemia and lymphoma panels. The Cyto-Chex diluted bone marrow sample results were compared to bone marrow samples collected in K2EDTA tubes. To date, we have tested 12 samples according to the presumptive clinical diagnosis of myelodysplastic syndrome, chronic lymphocytic leukemia, or acute myeloid leukemia. Results indicate that regardless of the leukemia and lymphoma panel type, the samples diluted in Cyto-Chex reagent tested at 6 hours and 72 hours yielded results phenotypically comparable to samples collected in K2EDTA tubes and tested at 6 hours. We conclude that bone marrow samples diluted in Cyto-Chex are stable for immunophenotyping analysis for up to 72 hours. In addition to bone marrow samples, laboratories have reported the use of Cyto-Chex for preservation of fine needle aspiration samples and other surgical biopsy samples.

Introduction

- Flow cytometric analysis of bone marrow samples has emerged as a critical component for proper diagnosis of Leukemias and Lymphomas.
- Frequently, samples have to be shipped from various clinics to the flow cytometry lab.
- The integrity of the bone marrow sample is critical for proper diagnosis.
- Extending the stability of bone marrow immune markers used for diagnosis of leukemias and lymphomas by flow cytometry would have great practical significance:
  - Personnel costs for weekend and evening samples can be reduced since staff need not work overtime or be on-call for routine flow cytometry analysis.
  - Extended stability would likely result in cost savings due to the processing of fewer expired samples.
  - Instrument setup/maintenance and processing time can be reduced by batching samples.
  - An especially important benefit of extended stability is eliminating the need for bone marrow redraws.
- In this study, we examine the use of the reagent, **Cyto-Chex®** (Streck Omaha, NE), for stabilizing bone marrow samples for 72 hours prior to flow cytometric analysis.
- Cyto-Chex diluted samples were compared to K2EDTA control samples for preservation of CD markers used in the diagnosis of leukemia and lymphoma.

Materials and Methods

- K2EDTA patient bone marrow samples were diluted 1:1 with Cyto-Chex reagent upon arrival at the flow cytometry laboratory.
- Cyto-Chex samples were stored at 4°C prior to testing.
- All samples were lysed using potassium chloride, washed and re-suspended with PBS + BSA + NaN3.
- Samples were stained with Beckman Coulter antibody using standard lyse wash protocol.
- After staining, samples were immediately run on the EPICS XL flow cytometer using System II software.

Figure 1: Representative dot plot of Cyto-Chex preserved patient bone marrow sample.

1A) 6 Hour Cyto-Chex CD45 vs. side scatter

1B) 3 Day Cyto-Chex CD45 vs. side scatter

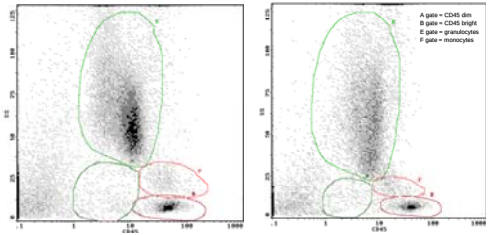


Table 1: Results of flow cytometry analysis of Lymphocyte gate (CD45 bright) in 5 bone marrow samples.

1A) Presumptive diagnosis and **diagnosis** of K2EDTA sample and Cyto-Chex 3 Day Sample.

Patient #	Presumptive Diagnosis	K2EDTA diagnosis	Cyto-Chex diagnosis
5	CLL	B-CLL	B-CLL
7	CLL	Normal	Normal
8	CLL	Normal	Normal
11	CLL	Normal	Normal
14	CLL	Normal	Normal

1B) Flow cytometry CD Marker **percent recovery** comparing K2EDTA sample vs. Cyto-Chex 3 Day Sample

5 Patients AVERAGES	K2EDTA 6 hour	Cyto-Chex 3 Day	% difference 3D Cyto-Chex Vs. K2EDTA
45+/14- %	98.4	92.0	-6.5
19+/3- %	6.3	5.3	-16.0
3+/19- %	66.3	61.0	-7.9
19+/20+ %	5.8	5.0	-13.0
19+/38+ %	2.0	2.0	0.0
19+/10+ %	2.0	2.0	0.0
5+/19+ %	2.0	2.0	0.0
5+/19- %	68.0	59.8	-12.1
19+/23+ %	2.0	1.6	-20.0
2+/7+ %	75.8	69.8	-7.9
3+/4+ %	38.0	34.3	-9.6
3+/8+ %	31.7	27.3	-13.7
19+/Kappa+ %	44.0	41.6	-5.5
19+/Lambda+ %	27.6	29.0	5.1

Table 2: Results of flow cytometry analysis of Blast gate (CD45 dim) in 7 bone marrow samples.

2A) Flow panel requested and **diagnosis** of K2EDTA sample and Cyto-Chex 3 Day Sample

Patient #	Presumptive Diagnosis	K2EDTA diagnosis	Cyto-Chex diagnosis
4	MDS	Normal	Normal
6	MDS	Normal	Normal
9	MDS	Normal	Normal
10	AML	CML	CML
12	MDS	Normal	Normal
13	MDS	Normal	Normal
15	MDS	Normal	Normal

2B) Flow cytometry CD marker **percent recovery** comparing K2EDTA sample vs. Cyto-Chex 3 Day Sample

7 Patients AVERAGES	K2EDTA 6 hour	Cyto-Chex 3 Day	% difference 3D Cyto-Chex Vs. K2EDTA
45+/14- %	93	90	-3.2
Total 33%	46	47	2.2
33+/13+ %	35	37	5.3
Total 34 %	31	29	-7.8
34+/13+ %	15	14	-3.8
Total HLA-DR %	50	46	-8.3
CD13+/HLA-DR+	23	21	-6.9
CD34+/CD117+	18	15	-15.3

Results

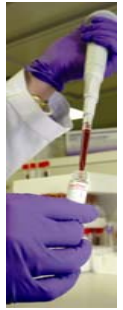
Cyto-Chex preserved the differentiation of bone marrow patient lymphocytes, monocytes, and granulocytes by CD45 vs. side-scatter on the flow cytometer.

Of the 12 Cyto-Chex-treated bone marrow patient samples examined at 6hr and 3 Day, all 12 had identical diagnoses as the 6hr K2EDTA sample.

Cyto-Chex-treated bone marrow samples gated on CD45 dim populations resulted in all 8 CD markers within 20% variation of the matched, fresh K2EDTA samples. Seven of the 8 CD markers displayed less than 10% difference from the values obtained from fresh K2EDTA samples.

Cyto-Chex-treated bone marrow samples gated on CD45 bright populations resulted in all 14 CD markers within 20% difference of the matched, fresh K2EDTA samples. Nine of the14 CD markers displayed less than 10% difference from the values obtained from fresh K2EDTA samples.

Conclusions



Cyto-Chex stabilized CD markers on the lymphocytes in bone marrow samples for up to 72 hours.

The flow cytometric diagnosis is unchanged when comparing Cyto-Chex-treated bone marrow samples and K2EDTA samples.

Use of Cyto-Chex greatly extends the time interval between specimen collection and analysis, offering improved patient convenience, the elimination of re-draws, and a cost savings for the laboratory.

Future Directions

Target the accrual of diseased bone marrow samples.

Test the efficacy of Cyto-Chex capability to preserve fine needle aspirates and tissues.

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