Streck Cell Preservative Preserves Fine Needle Aspiration Samples for Immunophenotyping by Flow Cytometry.

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Abstract:
Flow cytometry is an important analytical tool that, during the past 20 years, has become a widely accepted method to support diagnosis, establish prognosis, and assist in therapy choices for many hematological malignancies.

An important clinical application of flow cytometry is the immunophenotyping of unique expression of cell surface cytoplasmic markers. It has become a critical element for the diagnosis of hematological malignancies with the new WHO classification of leukemia and lymphoma. The classification requires lineage and immunologic features for the diagnosis, and when combined with fine needle aspiration, many cases of leukemia and lymphoma can be diagnosed easily without invasive open biopsies.

The delay in processing and storing flow cytometry samples is an unavoidable consequence of transportation from remote locations or from late night procedures. This situation will require the study of stability of both light scattering properties and cell surface markers on mononuclear cells from different types of flow cytometry samples prior to immunophenotyping.

Streck Cell Preservative is a storage solution for lymphoid tissue, bone marrow aspirates, whole blood or buffy coat preparations of peripheral blood mononuclear cells. This reagent has been used to successfully preserve cells of many origins for flow cytometry analysis. However, there is no current documentation describing the use of Streck Cell Preservative with fine needle aspirate (FNA) samples. The nature of this type of sample makes it difficult to obtain a sufficient volume of material for analysis. This technical brief explores the use of Streck Cell Preservative to preserve fine needle aspirations and body fluid specimens. The data presented establishes the effectiveness of Streck Cell Preservative for fine needle aspirations and body fluid specimens prior to flow cytometry analysis.

Materials and Methods:
Sample Preparation
The flow cytometry laboratory at Washington Hospital Center routinely processes and analyzes different types of surgically resected specimens for suspected diagnosis of leukemia and lymphoma. This study included difficult-to-obtain specimens and those with low cellularity including body fluids and diagnostic FNA material. Fine needle aspirations were performed on excised lymphoid tumors submitted to the Pathology Department for lymphoma flow cytometry analysis. Eight to ten FNA passes were performed on each specimen to recover cells for flow analysis.

Streck Cell Preservative Addition
The fresh material was minced in BioWhittaker™ X-Vivo balanced salt solution (Cambrex, East Rutherford, NJ) and the cell suspension was submitted to the flow cytometry laboratory for immunophenotyping, pathologist review and final reporting. For this study, excess sample was placed in Streck Cell Preservative (Streck) in a 1:1 (v/v) ratio. The samples were mixed well (>20 times) and held at 4-8°C for seven days. Flow cytometry was performed on these samples at 24 hours, 72 hours and 7 days. Flow cytometric results from fresh material were compared to those of the same sample preserved in Streck Cell Preservative.

Data Analysis
All data interpretations followed that of the CAP survey protocol. Briefly, FSC and SSC were examined for light scatter properties. Acceptable light scatter properties were defined as clear separation between lymphocytes, monocytes and debris. Light scatter properties were recorded as poor, good or excellent based on these criteria (Data not shown).

The intensity of fluorescence for each CD-marker was compared to that of the isotype negative controls. Cells with intensities equal to the isotype control of the same fluorochrome were considered negative. Intensities that were greater than the isotype control, but not one decade brighter were considered dimly positive. Intensities that were one decade greater than the isotype control were considered moderately positive. Cells with intensities greater than one decade from the isotype control were considered bright.

Dot-plot data of preserved samples was compared with the results obtained on the fresh sample in an effort to determine whether clinically significant changes had occurred due to the preservation. The supervising attending pathologist interpreted the histograms without knowledge of the final diagnosis rendered on fresh samples. The pathologist made a reasonable diagnosis based only on the FNA smear and the flow cytometry data. The blind was broken only after all data had been evaluated.

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Results:

The study was conducted on six cases that included five surgical specimens and one pleural fluid sample. Smears were prepared from fresh samples whenever possible. The flow cytometry data showed some changes in intensities and percentage of the positive cells. However, dot-plot data using the semi-quantitative intensity guidelines (Negative, Dimly Positive, Moderately Positive, Bright), did not result in significant changes.

The following diagnoses were obtained from the sample types listed in parentheses.

1. Reactive Follicular Hyperplasia (Neck mass)
2. Reactive Lymphoid Hyperplasia (Submandibular mass)
3. Reactive Lymphoid Hyperplasia (Lymph node)
4. B-cell Non-Hodgkin's Lymphoma, Follicular Center Cell Phenotype (Submandibular mass)
5. B-cell Non-Hodgkin's Lymphoma (Pleural fluid)
6. Reactive Lymphoid Hyperplasia (Lymph node)

Even though the resolution of the dot-plot data for some CD-markers or samples deteriorated over time, simple comparison with the original diagnosis showed a 100% concordance rate with all specimens after 24 hours, 72 hours and seven days storage in Streck Cell Preservative. Table 1 summarizes the results obtained using intensity guidelines described above.

Our data indicated that low-cellularity specimens did not preserve as well (data not shown), thus, cell counts and differentials for body fluids and smear reviews for body fluid and FNA are essential to examine prior to storage in Streck Cell Preservative.

Conclusions:

The data presented in this technical paper illustrates the utility of Streck Cell Preservative to preserve FNA and body fluid samples. This blinded study yielded patient diagnoses of 100% concordance between freshly prepared samples and samples stored in Streck Cell Preservative for seven days.

Streck Cell Preservative offers the laboratory greater flexibility for handling samples that readily deteriorate, like fine needle aspirations and body fluids. These types of specimens can be collected in the evening or over the weekend and stored in Streck Cell Preservative until the flow cytometry laboratory is available to process them. While the best laboratory practice is to process these samples immediately, circumstances arise that prohibit immediate analysis. The integrity of these samples can now be preserved with Streck Cell Preservative for up to seven days prior to analysis by flow cytometry.

Reactive Follicular Hyperplasia

Case	Analysis	CD45	CD14	CD7	CD10	CD3	HLA-DR	CD2	CD4	CD8	CD5	CD23	CD20	Kappa	Lambda	CD19	FMC-7	CD25
1 Neck Mass	Fresh																	
	24 hours																	
	72 hours																	
	7 days																	

Reactive Lymphoid Hyperplasia

Case	Analysis	CD45	CD14	CD7	CD10	CD3	HLA-DR	CD2	CD4	CD8	CD5	CD23	CD20	Kappa	Lambda	CD19	FMC-7	CD25
2 Submandibular Mass	Fresh																	
	24 hours																	
	72 hours																	
	7 days																	

Reactive Lymphoid Hyperplasia

Case	Analysis	CD45	CD14	CD7	CD10	CD3	HLA-DR	CD2	CD4	CD8	CD5	CD23	CD20	Kappa	Lambda	CD19	FMC-7	CD25
3 Lymph Node	Fresh																	
	24 hours																	
	72 hours																	
	7 days																	

B-cell Non-Hodgkin's Lymphoma

Case	Analysis	CD45	CD14	CD7	CD10	CD3	HLA-DR	CD2	CD4	CD8	CD5	CD23	CD20	Kappa	Lambda	CD19	FMC-7	CD25
4 Submandibular Mass	Fresh																	
	24 hours																	
	72 hours																	
	7 days																	

B-cell Non-Hodgkin's Lymphoma

Case	Analysis	CD45	CD14	CD7	CD10	CD3	HLA-DR	CD2	CD4	CD8	CD5	CD23	CD20	Kappa	Lambda	CD19	FMC-7	CD25
5 Pleural Fluid	Fresh																	
	24 hours																	
	72 hours																	
	7 days																	

Reactive Lymphoid Hyperplasia

Case	Analysis	CD45	CD14	CD7	CD10	CD3	HLA-DR	CD2	CD4	CD8	CD5	CD23	CD20	Kappa	Lambda	CD19	FMC-7	CD25
6 Lymph Node	Fresh																	
	24 hours																	
	72 hours																	
	7 days																	

* FNA Lymph node obtained during surgical procedure

Bright
Moderately Positive
Dimly Positive
Negative

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