Food and Drug Administration
Center for Biologics Evaluation and Research
Document Mail Center HFM-99 200N
1401 Rockville Pike
Rockville, MD 20852-1448

510(k) Summary of Safety and Effectiveness Information

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and CFR 807.92.

The assigned 510(k) number is _____

Address:	StemCo Biomedical, Inc. 2810 Meridian Parkway Suite 148 Durham, North Carolina 27713
Phone:	(919) 484-2571
Fax:	(919) 484-8792
Contact:	Cynthia Pritchard, PhD, extension 264 Director of Product Development OR N. Rebecca Haley, MD, extension 245 Medical Director, VP for Regulatory and Clinical Affairs

Date of Summary: September 30, 2004

Trade name: ALDECOUNT™

Common Name: ALDECOUNT

Classification Name: ALDECOUNT fits the description for the category of 'progenitor cell enumeration kit' which are Class II devices described as ancillary devices of 21 CFR Part 864.5220 (CFR Part 864 Hematology and Pathology Devices, Hematology analyzers), device code GKZ.

Predicate Device: The device is substantially equivalent to the originally cleared ALDECOUNT, BK030064.

Intended Use: ALDECOUNT is used to enumerate by flow cytometry low side scatter cells that express high levels of aldehyde dehydrogenase ($ALDH^{br}$ SSC^{lo}) in human peripheral blood, leukapheresis, and bone marrow samples. The test is for in vitro diagnostic use and is suited only for professional use in specialized clinical laboratories.

Indications for Use: This device is to be used for general diagnostic purposes to enumerate $ALDH^{bright}$ SSC^{lo} cells in fresh or frozen clinical samples. These samples can be from normal donors, donors mobilized with granulocyte colony stimulating factor (G-CSF) or some other mobilizing agent or patients that have been mobilized or treated with chemotherapeutic agents. This device is to be used by highly trained laboratory personnel skilled in cell staining and flow cytometry analysis.

Summary and Principle: $ALDH^{bright}$ SSC^{lo} cells have been shown to have properties of hematopoietic stem and progenitor (HSP) cells (Fallon et.al. 2003. Br. J. Hematology. 122: 99-108). Human cells with high ALDH activity become intensely fluorescent ($ALDH^{bright}$, $ALDH^{br}$) when exposed to ALDECOUNT reagent, a substrate for ALDH. The dry ALDECOUNT reagent is provided in a stable, inactive form. The activated form of ALDECOUNT freely diffuses into cells and is a non-toxic substrate for ALDH. The amount of fluorescent ALDH reaction product that accumulates in viable cells directly correlates to the ALDH activity in these cells.

This fluorescent substrate is converted by intracellular ALDH into a charged product that, under the conditions used for the reaction, is retained only in intact cells that express high levels of ALDH. Even if the cells are capable of transporting the substrate across the cell membrane and have active intracellular ALDH, cells that are apoptotic or nonviable have 'leaky' membranes and are not capable of retaining the charged product.

The product is intended for use with a flow cytometer equipped with a 488 nm argon ion laser for excitation and a 525 nm band pass filter. HSP cells are identified by flow cytometry as cells with low side scatter and high expression of ALDH. Such cells are recognized by comparing the fluorescence in a test sample to that in a control containing diethylaminobenzaldehyde (DEAB), a specific inhibitor of ALDH.

For use, ALDECOUNT is dissolved in dimethylsulfoxide, converted to the active substrate form by treatment with hydrochloric acid, and diluted to the working concentration with the ALDECOUNT neutralization buffer. To perform the assay, an aliquot of cells in assay buffer is added to the activated substrate. An aliquot of this cell mixture is immediately transferred to DEAB for the control. These mixtures are incubated to allow conversion of the substrate to the negatively charged product. The amount of intracellular fluorescent product can be measured by flow cytometry.

Comparison of ALDECOUNT to the Predicate Device:

Studies conducted at StemCo show that ALDECOUNT test conditions that were optimal for apheresis collections and peripheral blood samples are identical to optimal conditions for detection of $ALDH^{br}$ cells in bone marrow samples. The technological characteristics and principle of operation of ALDECOUNT have not changed from the original. The principles for use and assay controls are the same.

Comparison of new ALDECOUNT to the original ALDECOUNT (predicate method)

Name	ALDECOUNT	ALDECOUNT (original)
Company	StemCo Biomedical, Inc.	StemCo Biomedical, Inc.
510(k) #	TBD	BK030064
Class	II	Same
Code	GKZ	Same
Product Type	In vitro diagnostic kit	Same
Intended Use	to enumerate by flow cytometry low side scatter cells that express high levels of aldehyde dehydrogenase ($ALDH^{bright}$ SSC^{lo}) in human peripheral blood, leukapheresis and bone marrow samples	to enumerate by flow cytometry low side scatter cells that express high levels of aldehyde dehydrogenase ($ALDH^{bright}$ SSC^{lo}) in human peripheral blood and leukapheresis samples
Method	'Staining' reagent; fluorescent color	Same
Basis	Oxidation of fluorescent substrate by internal enzyme (aldehyde dehydrogenase)	Same
Signal	Fluorescence/scatter	Same
Instrument	Flow cytometer	Same
Control(s)		
Cell integrity	Intrinsic to assay	Same
Non-specific binding reaction	Enzyme inhibitor	Same
'gate'	primary gate is forward-scatter set to exclude debris and non-nucleated cells	Same
# of tubes per sample	Two (test and negative control)	Same
Format	Dry reagent; single test	Same; bulk format

Cross-reactivity	Discrimination intrinsic to assay by use of DEAB and side scatter	Same
Sensitivity, range	Linear from 0.1% to at least 18% $ALDH^{br}$ cells	Same
Sample	Human peripheral blood, apheresis, and bone marrow samples from normal donors, mobilized donors and patients, and patients treated with chemotherapeutic agents	Human peripheral blood and apheresis samples from normal donors, mobilized donors and patients, and patients treated with chemotherapeutic agents
Sample processing	erythrocyte lysis, wash, RT	Same
Reaction	30 minute incubation, 37°C	Same

Nonclinical Performance Data: Preclinical testing was done at StemCo using the ALDECOUNT kit to determine the performance characteristics of the system with marrow samples. Summary of the results with peripheral blood and apheresis collections are in the SSE for BK030064.

Time requirements: Activate precursor at room temperature for 15 minutes; do not exceed 35 minutes before addition of the neutralization buffer. Use activated substrate within 24 hr of activation. The reaction of substrate with cells from marrow samples can be done for 30 minutes (not to exceed 60 minutes) at 37°C.

Stability: The ALDECOUNT reagents are stable unopened at refrigerator temperatures or less (<8°C) for at least two years.

Sensitivity/dynamic range: linear range is 0.1% to at least 17%; marrow samples tested to date contained 0.1 to 5% $ALDH^{br}$ cells.

Precision: coefficient of variation (CV) $\leq$5% with samples containing at least 1% $ALDH^{br}$ cells, $\leq$20% with samples containing 0.1% $ALDH^{br}$ cells

Interferences: Phenotyping with monoclonal antibodies and viability dyes is compatible with the ALDECOUNT assay with marrow samples. Erythrocyte lysis buffers that contain detergent or fixatives will interfere in the assay. Addition of ALDECOUNT substrate to cells or performance of the ALDECOUNT assay does not interfere with viability of cells from marrow samples.

Clinical Performance Data: Initial trials were done with human clinical samples obtained from two major medical centers and from Cambrex (Gaithersburg, MD). These samples included peripheral blood and apheresis samples from normal donors, donors and patients mobilized with granulocyte colony stimulating factor, and patients treated with chemotherapeutic agents. See summary for BK030064.

Additional clinical studies were done at three major medical centers and at StemCo, in which ALDECOUNT in a single-test format was compared to ProCOUNT and to the ISHAGE method using CD34-APC, CD45-PE, and 7-AAD. Samples in these studies included fresh and previously frozen peripheral blood and apheresis samples from

normal donors, donors and patients mobilized with granulocyte colony stimulating factor, and cancer patients treated with various chemotherapeutic agents. Comparison of results from each of the assays at each site and combined results with Student's t Test showed no significant differences between the means in any case. Linear regression analysis of results from ALDECOUNT and ProCOUNT and from ALDECOUNT and ISHAGE produced acceptable slopes and coefficients of determination (see table below).

Comparison	Site; N	Linear Regression Analysis		t Test
ALDECOUNT to ProCOUNT		R^2	Slope	p =
	1; 29	0.98	1.08	0.88
	2; 57	0.85	0.92	0.42
	3; 10	0.82	0.93	0.76
	Combined; 96	0.90	0.96	0.88
ALDECOUNT to ISHAGE	1; 65	0.99	1.11	0.85
	2; 57	0.96	0.81	0.18
	3; 40	0.99	0.96	0.90
	Combined; 162	0.99	1.07	0.35

Enumerating $ALDH^{br}$ cells in bone marrow samples with ALDECOUNT yields results similar to those observed with peripheral blood and apheresis samples. Results of assays with bone marrow performed to date show a correlation (R^2) of 0.96 and a slope of 0.94, indicating that there is no effect of additional antibody conjugates or viability marker (7-AAD exclusion dye) on the ALDECOUNT results. Comparison of total and viable $ALDH^{br}$ cells in bone marrow samples produced a correlation (R^2) of 0.97 and a regression line slope of 1, indicating few, if any, nonviable $ALDH^{br}$ cells. Average values and ranges of $ALDH^{br}$ cells (1.1%; 0.14 to 4.38%) and viable CD34+ cells (1.3%; 0.34 to 3.5%) observed in bone marrow samples were similar. Nonviable (7-AAD+) CD34+ cells were kept to a minimum (average 13%) by using only fresh samples tested within 48 hours of collection. With marrow samples, the ALDECOUNT assay produced linear results from 0.1% to at least 5% $ALDH^{br}$ cells.