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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 BK030064.

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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 a number of devices that have a similar intended use, such as Becton Dickinson's ProCOUNT (BK960087; see below).

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 and leukapheresis samples, and $ALDH^{br}$ cells in 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 ($ALDH^{bright}$, $ALDH^{\mathbf{br}}$) 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:

ProCOUNT uses a conjugated CD34 antibody to stain progenitor cells and determines the number of these by flow cytometry. CD34 is one "cluster differentiation" marker used to identify stem/progenitor cells, whereas ALDECOUNT uses a substrate to 'stain' for an enzyme found in high levels in stem/progenitor cells. Both of these markers identify a population of cells with low side scatter by flow cytometry. ProCOUNT 'gates' the side scatter through either a CD45+ cell gate or through a total nucleated cell gate, two methods that produce identical results. The ALDECOUNT method uses forward scatter as the primary gate to eliminate debris and non-nucleated cells. See the table below for a comparison of the StemCo method to this device.

Though the technological basis for the methods is different, the correlation of results produced with fresh clinical samples by ProCOUNT and ALDECOUNT was excellent. Monoclonal antibody-based methods detect both viable and nonviable cells, whereas ALDECOUNT detects only intact cells. The percentages of dead or damaged cells were kept to a minimum by using only fresh samples in the clinical studies described in the submission.

Comparison of ALDECOUNT to the original predicate method

Parameter	Device	Predicate Device
Name	ALDECOUNT	ProCOUNT Progenitor Cell Enumeration Kit
Company	StemCo Biomedical, Inc.	Becton Dickinson, Inc.
510(k) #	BK030064	BK960087
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 and leukapheresis samples and $ALDH^{br}$ cells in bone marrow cells	To identify and enumerate absolute counts and percentages of CD34+ cells in human peripheral blood samples, mobilized peripheral blood and leukapheresis samples using flow cytometry
Method	'Staining' reagent; fluorescent color	Same
Basis	Oxidation of fluorescent substrate by internal enzyme (aldehyde dehydrogenase)	Fluorescence-conjugated monoclonal antibody to CD34 surface antigen

Signal	Fluorescence/scatter	Same
Instrument	Flow cytometer	Same
Control(s)		
Cell integrity	Intrinsic to assay	Indirect, uses a nucleic acid intercalating dye to detect nucleated cells
Non-specific binding reaction	Enzyme inhibitor	Antibody isotype control
'gate'	primary gate is forward-scatter set to exclude debris and non-nucleated cells	Nucleated cells or CD45+ cells (produce identical results)
# of tubes per sample	Two (test and negative control)	Same
Format	Dry reagent	Dry particles, liquid reagents
Cross-reactivity	Discrimination intrinsic to assay by use of DEAB and side scatter	No cross-reactivity of CD34 in non-progenitor cells in blood from normal donors
Sensitivity, range	0 to 3000 $ALDH^{br}$ cells per microliter	0 to 2000 CD34+ cells per microliter
Sample	Human peripheral blood, apheresis, and bone marrow samples from normal donors and 'mobilized' donors and patients	Same
Sample processing	erythrocyte lysis, wash, RT	Same, except sample is treated to lyse erythrocytes after staining
Reaction	30 to 60 minute incubation, 37^{o}C	Same

Nonclinical Performance Data: Preclinical testing was done at StemCo using the ALDECOUNT kit to determine the performance characteristics of the system.

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 can be done from 30 to 60 minutes at 37^{o}C.

Stability: The ALDECOUNT reagents are stable unopened at refrigerator temperatures or less (<8^{o}C) for at least two years. Freezing of the reagents has no effect on assay performance.

Sensitivity/dynamic range: linear range is at least 0.1% to 18% $ALDH^{br}$ cells in a background of 10^6 leukocytes per ml

Precision: coefficient of variation (CV) $\leq$5% at high end of range, $\leq$20% at low end of range (CV determined from at least 40 replicates), based on percentages of $ALDH^{br}$ cells

Interferences: Phenotyping with monoclonal antibodies and viability dyes is compatible with the ALDECOUNT assay. Peripheral blood can be drawn into heparin, ACD-A, or EDTA anticoagulants; samples can be stored at 2 to 8^{o}C for up to 48 hours with no effect on results. Filling of 5 ml blood collection tubes with 3 to 5 ml had no effect on results. 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 cell viability or clonogenicity.

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, and donors and patients mobilized with granulocyte colony stimulating factor. Samples (N = 72) were tested using the ALDECOUNT Assay and ProCOUNT at two different sites. Linear regression analysis of CD34+ and $ALDH^{br}$ cells produced a slope of 1.1 at both sites and a coefficient of determination of 0.965 and 0.944 from the two sites, indicating that the basic technological difference in these staining methods does not affect the end result and that these methods produce similar results for this type of application. These results were corroborated with a modified ISHAGE protocol using CD34-APC, CD45-PE, and 7-AAD, which produced a slope of 0.93 with a coefficient of determination of 0.96 in comparisons with ALDECOUNT on 388 fresh and frozen clinical samples.

Additional clinical studies were done at three major medical centers and at StemCo, in which ALDECOUNT 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).

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. These same marrow samples were stained in parallel with the modified ISHAGE method; results showed that up to 75% of the CD34+ cells were nonviable in these samples. With marrow samples, the ALDECOUNT assay produced linear results from 0.1% to at least 5% $ALDH^{br}$ cells.

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

Limitations

ALDECOUNT will detect only cells with intact membranes that express high levels of ALDH. Therefore, ALDECOUNT results will correlate highly with anti-CD34 monoclonal antibody-based methods to detect stem and progenitor cells only if immunophenotyping is done in conjunction with membrane exclusion dyes such as 7-AAD.

This product has not been tested with pediatric samples.

Samples from non-mobilized donors and patients do not produce high correlations between $ALDH^{br}$ and CD34+ cells in regression analysis (slopes = 0.8, R2 = 0.94 and 0.84, respectively).