ITEM 7. 807.87(h). A 510(k) SUMMARY AS DESCRIBED IN 807.92 OR A 510(k) STATEMENT AS DESCRIBED IN 807.93.

K935720

MAY -9 1996

510(k) SUMMARY

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DEVICE NAME: Ortho-Count Calibration Kit for ORTHO CYTORONABSOLUTE™ Laser Flow Cytometry System

[864.8185 Calibrator for red cell and white cell counting]

PREDICATE: S-CAL® Kit [K813375] Coulter Diagnostics

DATE: November 29, 1993

DEVICE DESCRIPTION:

Ortho-Count Calibration Kit for ORTHO CYTORONABSOLUTE Laser Flow Cytometry System, contains a Calibrator Bead Suspension for absolute lymphocyte count calibration and Verifier Bead Suspensions (I, II and III) to verify accuracy of absolute count calibration.

The Calibrator Bead Suspension is manufactured as known "events"/unit volume (i.e. the Calibration Number) in the form of microparticle beads suspended in a proprietary buffer. The size of the microparticles has been selected to emulate the range of size of leukocytes detected by the ORTHO CYTORONABSOLUTE under specified gating conditions.

The Verifier Bead Suspensions (I, II and III), are composed of materials identical to the Calibrator Bead Suspension and collectively provide a range of particle bead concentrations. The target values chosen approximate values expected from patients with low (Verifier I), normal (Verifier II) and high (Verifier III) white blood cell counts, respectively. The Verifiers are used to verify accuracy of the absolute lymphocyte count calibration immediately following calibration and are also used as daily quality control verification that the ORTHO CYTORONABSOLUTE is in calibration. A properly calibrated instrument will provide absolute counts within ± 10% of the labeled events/uL for each Verifier supsension.

In the context of immune status monitoring, absolute cell count is defined as the number of cells per unit volume. The ORTHO CYTORONABSOLUTE uses precision stepper motors to deliver diluted sample to the instrument's flow cell at a constant rate. Since constant flow rate means movement of a fixed volume per unit time, analysis time determines the volume of sample analyzed. Calibration of the ORTHO CYTORONABSOLUTE consists of setting analysis time such that a volume corresponding to 1 uL of undiluted sample is examined.

Ortho-Count Calibrator Bead Suspension, representing a known number of particles per unit volume (i.e. events/uL) at the same dilution as used for testing samples, is counted until its "Calibration Number" is reached; the instrument is set to this analysis time. In subsequent analyses events are counted for this predetermined period of time, now calibrated to correspond to passage of the equivalent of 1 uL of undiluted sample through the instrument flowcell. As a result of this calibration, sample results can be read in absolute count, i.e. "events"/uL.

INTENDED USE:

Ortho-Count Calibration Kit for ORTHO CYTORONABSOLUTE Laser Flow Cytometry System is intended to be used to calibrate and verify calibration of the absolute lymphocyte counting function of the ORTHO CYTORONABSOLUTE. This intended use is substantially equivalent to the intended use of S-CAL Kit, the predicate device, for lymphocyte counting. The intended use is different than that for S-CAL Kit in that Ortho-Count Calibration Kit may not be used to calibrate the ORTHO CYTORONABSOLUTE for parameters other than lymphocyte counting. This difference does not affect the safety or effectiveness of Ortho-Count Calibration Kit for its intended use since the ORTHO CYTORONABSOLUTE is not intended for all of the same uses as the Coulter Systems with which S-CAL Kit is intended to be used.

TECHNOLOGICAL CHARACTERISTICS:

Ortho-Count Calibration Kit and S-CAL Kit both contain components which are recognized by cell counters as leukocytes and are used to convert the electronic signal obtained from such instruments into accurate leukocyte count results expressed in clinical terms. Both kits consist of suspensions of particles, whose size, shape, concentration and other characteristics have been accurately determined. Ortho-Count Calibration Kit employees microparticle beads, while S-CAL Kit uses fixed erythrocytes in suspension, to simulate leukocytes.

PERFORMANCE DATA:

Performance of an ORTHO CYTORONABSOLUTE calibrated using Ortho-Count Calibration Kit (Meth 2) was compared to performance of the same instrument calibrated using whole blood whose total lymphocyte count was determined with a Coulter JT2 calibrated using S-CAL Kit (Meth 1), and to performance of the Coulter JT2 calibrated with S-CAL Kit (JT2). As shown in Table 1, there were no significant differences in absolute lymphocyte counts that could be attributed to either the instrument or the method of calibration.

TABLE 1

LYMPHOCYTE COUNTS FROM A HEMATOLOGY ANALYZER VERSUS

LYMPHOCYTE COUNTS FROM ORTHO CYTORONABSOLUTE CALIBRATED BY

DIFFERENT METHODS

Donor Number	Mean JT2	Std. Dev.	Mean Method 1	Std. Dev	Mean Method 2	Std. Dev	Ratio Meth1 JT2	Ratio Meth2 JT2	Ratio Meth2 Meth1
1	1983	75	1776	23	1940	90	0.90	0.98	1.09
2	2383	41	2189	94	2147	88	0.92	0.90	0.98
3	1767	137	1736	71	1668	93	0.98	0.94	0.96
4	2217	75	2076	81	2129	90	0.94	0.96	1.03
5	1800	47	1805	52	1807	110	1.00	1.00	1.00
6	2250	55	2265	29	2132	56	1.01	0.95	0.94
7	1600	122	1557	25	1476	98	0.97	0.92	0.95
8	2300	71	2323	138	2271	61	1.01	0.99	0.98
9	3820	164	3730	81	3855	94	0.98	1.01	1.03
10	2517	117	2481	73	2413	141	0.99	0.96	0.97
				Mean			0.97	0.96	0.99
				Standard Deviation			0.04	0.04	0.05

Four ORTHO CYTORONABSOLUTE instruments located in different regions of the United States, were calibrated using Ortho-Count Calibration Kit to assess inter-laboratory reproducibility as evidenced by Ortho-Count Calibration Kit Verifier Bead Suspensions. **Table 2** shows the accuracy and precision of the absolute counts at each site. Overall precision of the absolute counts between laboratories was equivalent to the within laboratory precision.

Table 2. Across Site Accuracy and Precision of Absolute Counts on Four Microparticle Calibrated CytoronAbsolutes

Location	Site One	Site Two	Site Three	Site Four	Overall Inter-Lab
Verifier I					
Mean	1736	1699	1669	1670	1693
Std. Dev.	61	43	43	44	55
% CV	3.5	2.5	2.6	2.6	3.2
Verifier II					
Mean	7851	7726	7805	7614	7748
Std. Dev.	174	225	101	173	193
% CV	2.2	2.9	1.3	2.3	2.5
Verifier III					
Mean	15434	15169	15209	14755	15140
Std. Dev.	257	492	225	288	409
% CV	1.7	3.2	1.5	2.0	2.7

Consistency of calibration of ORTHO CYTORONABSOLUTE instruments was demonstrated in a study comparing absolute lymphocyte counts obtained on a panel of donors at each of the four study sites.

Mean absolute lymphocyte counts from eight determinations on each donor per site were plotted against the overall mean lymphocyte count for each donor across sites (Figure 1).

Figure 1. Absolute Lymphocyte Counts Measured on Cross-Calibrated ORTHO CYTORONABSOLUTE Instruments

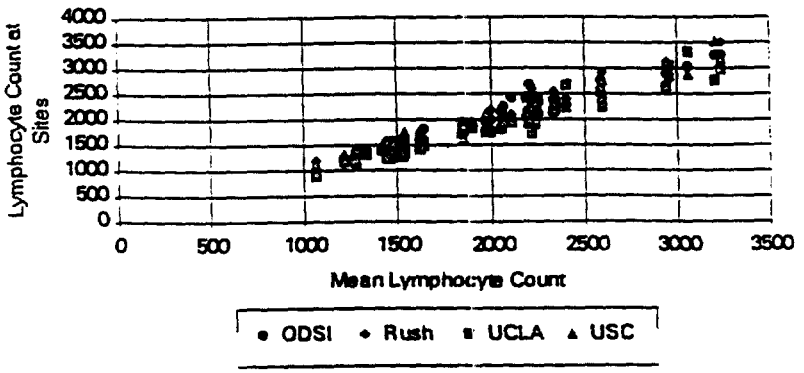

Figure 1. Absolute lymphocyte counts were determined using cross-calibrated ORTHO CYTORONABSOLUTE instruments for a whole blood panel kept overnight at room temperature or shipped for next day delivery to each site. The average lymphocyte count obtained at each site is plotted versus the mean lymphocyte count across sites for each of 41 samples.

The determination of an absolute lymphocyte subset count requires both a lymphocyte count and the percentage of lymphocytes positive for the marker. By convention, these measurements have required separate flow cytometry and hematology instruments. A study was performed to demonstrate that when calibrated using Ortho-Count Calibration Kit it is possible to obtain equivalent absolute lymphocyte subset counts directly from the ORTHO CYTORONABSOLUTE. Figure 2 shows strong correlation between absolute count values determined directly on the ORTHO CYTORONABSOLUTE and those calculated from combined hematology and other flow cytometer/reagent results. Regression analysis of the data yields a slope of 0.89, a y-intercept of -31 ± 13 and a correlation coefficient (r) = 0.96.

Figure 2. Direct Determination of Absolute Lymphocyte Counts on the ORTHO CYTORONABSOLUTE Versus Hematology Plus Other Flow Cytometer Calculations

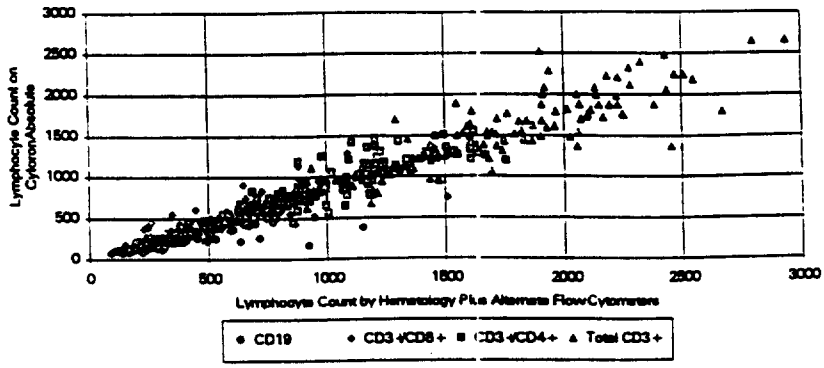

Figure 2. Absolute lymphocyte subset values were determined directly using a single ORTHO CYTORONABSOLUTE. These data are plotted versus absolute lymphocyte counts determined using separate flow cytometers and automated hematology analyzers. Absolute lymphocyte subsets plotted are: CD19, CD8+ cells that are also CD3+, CD4+ cells that are also CD3+ and total CD3+ cells

The utility of the Ortho-Count Calibrator Kit Verifier Bead Suspensions as quality control indicators of proper calibration of the ORTHO CYTORONABSOLUTE was demonstrated in a study in which counts/volume (absolute counts) were measured on the three verifier suspensions twice per week over a period of 60 days. Figure 3 shows the absolute counts obtained at each laboratory over the two months. The tight agreement in measurements made using each of the Verifier Bead Suspensions confirms the suitability of Ortho-Count Calibration Kit as an accurate quality control measure of the status of calibration of the ORTHO CYTORONABSOLUTE.

Figure 3. Ortho-Count Calibration Kit Verifier Bead Suspensions as Quality Control Indicators of Status of Calibration of the ORTHO CYTORONABSOLUTE

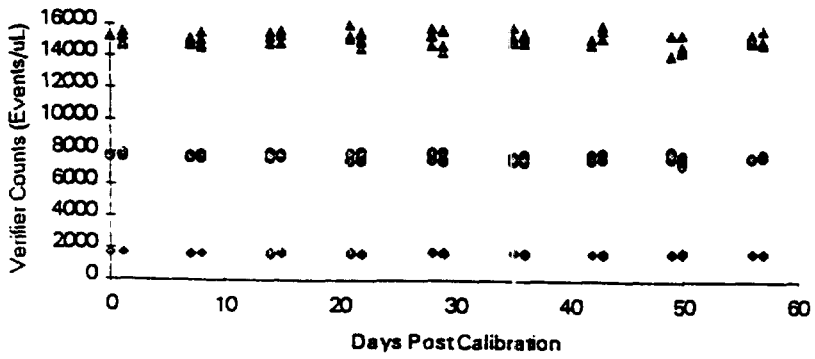

Figure 3. Four ORTHO CYTORONABSOLUTE instruments were calibrated using Ortho-Count Calibration Kit. The absolute count of each Verifier Bead Suspension is plotted for each laboratory on each day throughout the study.

CONCLUSIONS:

1. Ortho-Count Calibration Kit is substantially equivalent to S-CAL Kit used to calibrate Coulter hematology instruments, for the intended use of calibration and verification of the calibration of the absolute lymphocyte counting function of the ORTHO CYTORONABSOLUTE.

2. Ortho-Count Calibration Kit functions as a suitable cross-calibration standard to ensure inter-laboratory reproducibility.

3. Ortho-Count Calibration Kit provides accurate calibration and verification of calibration of the ORTHO CYTORONABSOLUTE, permitting direct determination of absolute lymphyocyte subset values equivalent to those obtained using values calculated from separatte flow cytometers and hematology analyzers.

4. Ortho-Count Calibration Kit provides reliable quality control of the calibration status of the ORTHO CYTORONABSOLUTE.