

K950482

REVISED 510(k) SUMMARY

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DEVICE NAME: Ortho-mune™ OK-COMBO
CD3-FITC/CD8-PE
(OKT™3/OKT8)
Monoclonal Antibody (Murine)

PREDICATE: Ortho-mune™ OKT™3
Monoclonal Antibody (Murine)
FITC Conjugate

Ortho-mune OKT8
Monoclonal Antibody (Murine)
Phycoerythrin Conjugate

DATE: February 29, 1996

DEVICE DESCRIPTION

Ortho-mune OK-COMBO CD3-FITC/CD8-PE (OKT3/OKT8) Monoclonal Antibody (Murine) is a blend of the individual purified monoclonal antibodies OKT3 and OKT8 conjugated to the fluorochromes fluorescein isothiocyanate and phycoerythrin respectively.

INTENDED USE

Ortho-mune OK-COMBO CD3-FITC/CD8-PE is intended for use in identification and enumeration of CD3+ and CD8+ human T lymphocytes in whole blood by flow cytometry. The intended use is the same as the intended use of the predicate device(s), Ortho-mune OKT3 Monoclonal Antibody (Murine) FITC Conjugate, and Ortho-mune OKT8 Monoclonal Antibody (Murine) Phycoerythrin conjugate.

TECHNOLOGICAL CHARACTERISTICS

Ortho-mune OK-COMBO CD3-FITC/CD8-PE (OKT3/OKT8) Monoclonal Antibody (Murine), Ortho-mune OKT3 Monoclonal Antibody (Murine) FITC Conjugate, and Ortho-mune OKT8 Monoclonal Antibody (Murine) Phycoerythrin Conjugate all utilize

Ortho Diagnostic Systems Inc.

Ortho-mune OK-COMBO CD3-FITC/CD8-PE Ref. No. K95-0482

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monoclonal antibodies specific for human T cells (OKT3) and human suppresser/cytotoxic T cells (OKT8) respectively. The purified monoclonal antibodies are conjugated to the same fluorochromes, fluorescein isothiocyanate and phycoerythrin respectively.

PERFORMANCE CHARACTERISTICS

Performance of the two color reagent Ortho-mune OK-COMBO CD3-FITC/CD8-PE (OKT3/OKT8) Monoclonal Antibody (Murine) was compared with that of single color reagents Ortho-mune OKT3 Monoclonal Antibody (Murine) FITC Conjugate and Ortho-mune OKT8 Monoclonal Antibody (Murine) Phycoerythrin Conjugate. Whole blood specimens from 199 normal donors, and 77 AIDS/ARC patients were stained and analyzed using the ORTHO CYTORONABSOLUTE™ flow cytometer, Ortho Diagnostic Systems Inc.

For each specimen, the percentage of gated cells which showed positive by each marker was calculated. The mean and range of the total percent CD3+ and total percent CD8+ for the normal population and the AIDS/ARC population are shown in Table 1 and Table 2, respectively.

TABLE 1

PERCENT POSITIVE STAINED CELLS IN NORMAL DONORS DETECTED BY OKT3/OKT8 AND OKT3 FITC AND OKT8 PE ASSAYED ON THE CYTORONABSOLUTE N=199					
Dual Color Reagent	Mean %	Range %	Single Color Reagent	Mean %	Range %
CD3+ (OKT3)	74.5	45.9-87.4	CD3+ (OKT3)	75.9	58.4-90.1
CD8+ (OKT8)	31.2	15.0-74.9	CD8+ (OKT8)	29.2	13.2-75.5

TABLE 2

PERCENT POSITIVE STAINED CELLS IN AIDS/ARC PATIENTS DETECTED BY OKT3/OKT8 AND OKT3 FITC AND OKT8 PE ASSAYED ON THE CYTORONABSOLUTE N=77					
Dual Color Reagent	Mean %	Range %	Single Color Reagent	Mean %	Range %
CD3+ (OKT3)	73.5	27.5-90.7	CD3+ (OKT3)	77.7	35.6-96.4
CD8+ (OKT8)	59.1	25.6-89.8	CD8+ (OKT8)	60.3	18.1-92.1

Linear regression analysis of total percent CD3+ cells from the combined normal and AIDS/ARC populations is found in Chart 1. Likewise, linear regression analysis of total percent CD8+ cells from the combined normal and AIDS/ARC populations is found in Chart 2.

CHART 1

OK-COMBO CD3/CD8 vs Ortho-mune OKT3 FITC

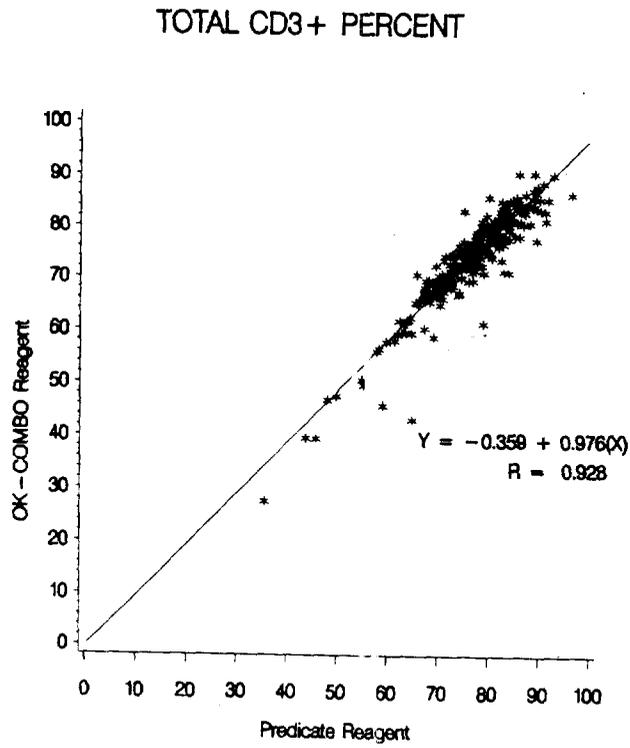
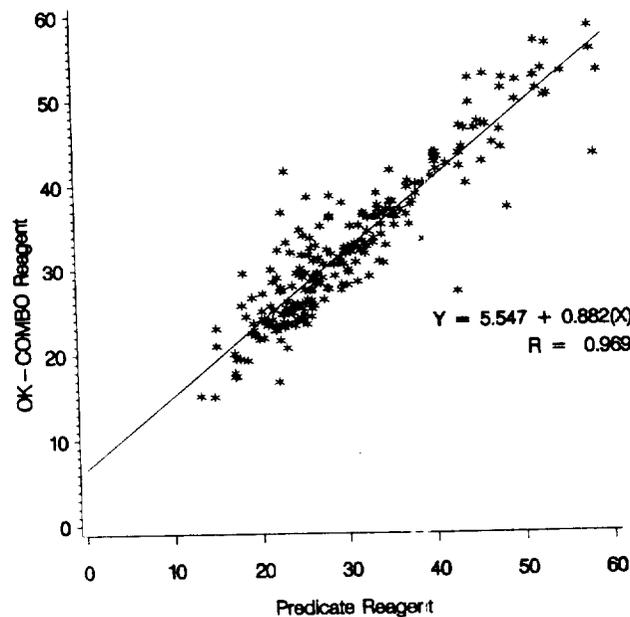


CHART 2

OK-COMBO CD3/CD8 vs Ortho-mune OKT8 PE

TOTAL CD8+ PERCENT



These studies demonstrate that the performance of the two color reagent Ortho-mune OK-COMBO CD3-FITC/CD8-PE (OKT3/OKT8) Monoclonal Antibody (Murine) is equivalent to the single color reagents Ortho-mune OKT3 FITC Conjugate and OKT8 PE Conjugate for determination of total percent CD3+ and total percent CD8+ cells in whole blood by flow cytometry.

For the CD3+CD8+ cell population the mean percent and range of results for the normal and AIDS/ARC population obtained using Ortho-mune OK-COMBO CD3-FITC/CD8-PE are shown in Table 3. Cells positive for both markers could not be determined with the single color reagents.

TABLE 3

PERCENT MEAN AND RANGE OF CD3+CD8+ CELLS IN NORMAL AND AIDS/ARC DONORS DETECTED BY OKT3/OKT8 ASSAYED ON THE CYTORONABSOLUTE					
Normal Donors	Mean %	Range %	AIDS/ARC Donors	Mean %	Range %
CD3+CD8+ (OKT3/OKT8)	25.8	12.3-67.5	CD3+CD8+ (OKT3/OKT8)	53.3	5.1-86

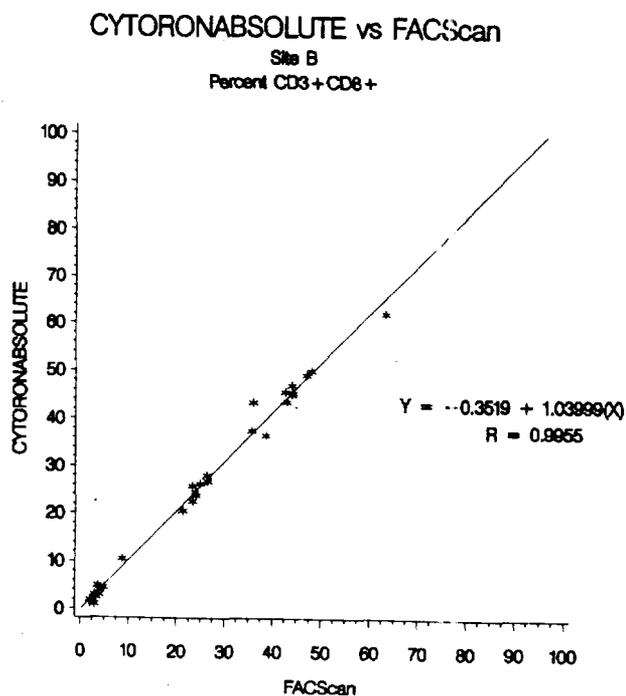
ORTHO CYTORONABSOLUTE™ Laser Flow Cytometer was used in the clinical studies comparing the performance of Ortho-mune OK-COMBO CD3-FITC/CD8-PE immunophenotyping reagent to Ortho-mune OKT3 FITC conjugate and OKT8 Phycoerythrin conjugate

A study was done comparing the performance of the ORTHO CYTORONABSOLUTE flow cytometer to recognized flow cytometers, Becton Dickinson FACScan™ and COULTER EPICS™ flow cytometers.

Specimens from ten normal donors (whole blood, EDTA) were processed using monoclonal antibodies bound to magnetic microbeads to product samples with low, normal, and high relative percent positive CD8 cells. A portion of each sample was stained in triplicate using immunophenotyping reagents from each manufacturer. Specimens stained with Ortho-mune OK-COMBO reagents were analyzed using the ORTHO CYTORONABSOLUTE flow cytometer. Likewise, samples stained with Simultest reagents were analyzed using the FACScan flow cytometer, and samples stained with Cyto-Stat®/ COULTER CLONE® were analyzed using the COULTER EPICS flow cytometer.

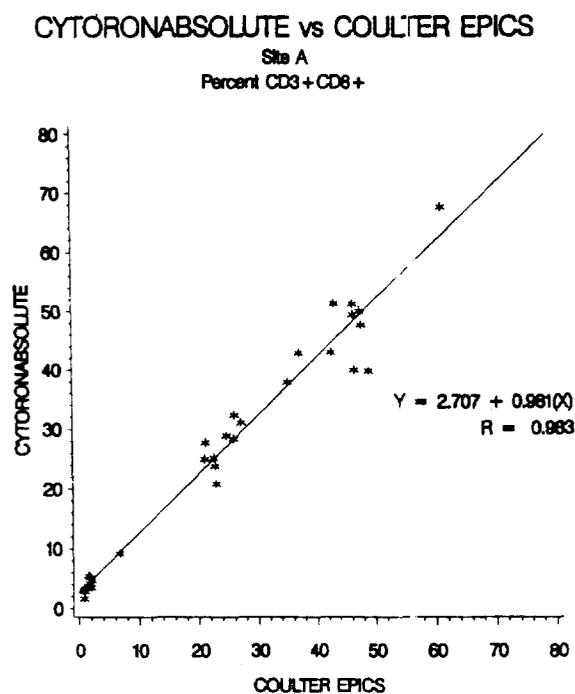
For each sample the mean of the triplicate results were calculated for the Becton Dickinson FACScan, COULTER EPICS, and CYTORONABSOLUTE within concentration and donor. Least square regression analyses were performed on these mean results for the FACScan (X axis) versus the CYTORONABSOLUTE (Y axis), and for the COULTER EPICS (X axis) versus the CYTORONABSOLUTE (Y axis). Results for percent CD3+CD8+ cells using Ortho-mune OK-COMBO CD3-FITC/CD8-PE and the CYTORONABSOLUTE versus Simultest CD3/CD8 (Leu-4/2a) using the FACScan are presented in Chart 3.

CHART 3



Results for percent CD3+CD8+ cells using Ortho-mune OK-COMBO CD3+CD8+ cells and the CYTORONABSOLUTE flow cytometer versus Cyto-Stat/ COULTER CLONE CD3(IgG1)-FITC/T8-RD1 and the COULTER EPICS flow cytometer are presented in Chart 4.

CHART 4



These studies demonstrate the equivalent performance of the ORTHO CYTORONABSOLUTE flow cytometer to both the Becton Dickinson FACScan and the COULTER EPICS flow cytometer for determination of percent positive cells when used with appropriate immunophenotyping reagents.

Reproducibility studies were performed at three independent laboratories using samples with low, normal, and high relative percent CD3+ and CD8+ cells.

Specimens from each of eleven normal donors (whole blood, EDTA) were processed using monoclonal antibodies bound to microbeads to produce samples of low, normal, and high relative percent CD3+ and CD8+ cells. The samples were separated into aliquots for each laboratory. Samples were stained in replicates of 10 with Ortho-mune OK-COMBO CD3/CD8 reagent and analyzed using the ORTHO CYTORONABSOLUTE flow cytometer.

For within laboratory reproducibility, the variance for the replicate results was calculated within site, concentration and donor. The variance was averaged across site, concentration and donor. The square root replicate variance (SD) was divided by the appropriate mean percent positive result (by site and concentration) and multiplied by 100 to obtain the CV. Within laboratory reproducibility results for determination of total percent CD3+ and percent CD3+CD8+ cells are presented in Table 4.

TABLE 4

WITHIN LABORATORY REPRODUCIBILITY OK-COMBO CD3/CD8							
N = 11 donors							
OK-COMBO CD3/CD8	All SITES Mean Percent Positive	Site A		Site B		Site C	
		CV	# Reps	CV	# Reps	CV	# Reps
TOTAL CD3+ Low	56.525	3.400	98	2.486	110	3.488	109
TOTAL CD3+ Normal	76.676	2.130	110	1.569	110	2.307	109
TOTAL CD3+ High	87.146	2.407	109	1.539	110	2.424	110
CD3+ CD8+ Low	4.010	11.214	108	9.430	110	11.151	104
CD3+ CD8+ Normal	28.053	4.793	110	3.610	110	5.250	109
CD3+ CD8+ High	44.311	4.359	98	2.904	110	4.147	109

The between laboratory CV was computed as follows. The mean percent positive for each site within concentration was calculated. The SD was computed on the three site means within concentration and the CV was obtained by dividing the SD by the overall mean within concentration and multiplying by 100. Between laboratory reproducibility results for determination of total percent CD3+ and percent CD3+CD8+ cells are presented in Table 5.

TABLE 5

BETWEEN LABORATORY REPRODUCIBILITY					
OK-COMBO CD3/CD8					
N = 11 donors					
OK-COMBO CD3/CD8	SITE A	SITE B	SITE C	ACROSS SITE	
	Mean Percent Positive (All Donors)	Mean Percent Positive (All Donors)	Mean Percent Positive (All Donors)	Coefficient of Variation	# Reps
TOTAL CD3⁺ Low	56.707	58.151	54.879	2.899	317
TOTAL CD3⁺ Normal	77.261	77.105	75.676	1.139	329
TOTAL CD3⁺ High	87.964	87.987	85.491	1.646	329
CD3⁺ CD8⁺ Low	4.088	3.467	4.439	12.309	322
CD3⁺ CD8⁺ Normal	28.451	27.058	28.614	3.051	329
CD3⁺ CD8⁺ High	45.009	44.386	43.396	1.837	317

Ortho-mune OK-COMBO CD3/CD8 immunophenotyping reagent shows acceptable within and between laboratory reproducibility for determination of total CD3+ and CD3+CD8+ lymphocyte percentages.

A linearity study was performed using an automated hematology analyzer to determine total lymphocyte count, and the CYTORONABSOLUTE flow cytometer to determine the percent positive CDx cells.

Specimens from four normal donors (whole blood, EDTA) were processed to produce samples with low, normal and high numbers of lymphocyte subsets. Each whole blood specimen was concentrated by harvesting the buffy coat to obtain a white blood cell count between 20,000 and 40,000 cells/ul and then diluting to produce samples of high, normal and low numbers of lymphocyte subsets. A portion of each sample was stained in triplicate using Ortho-mune OK-COMBO CD3/CD8 immunophenotyping reagent and analyzed using the CYTORONABSOLUTE flow cytometer. The total lymphocyte count of the concentrated sample for each donor was obtained using an automated hematology analyzer.

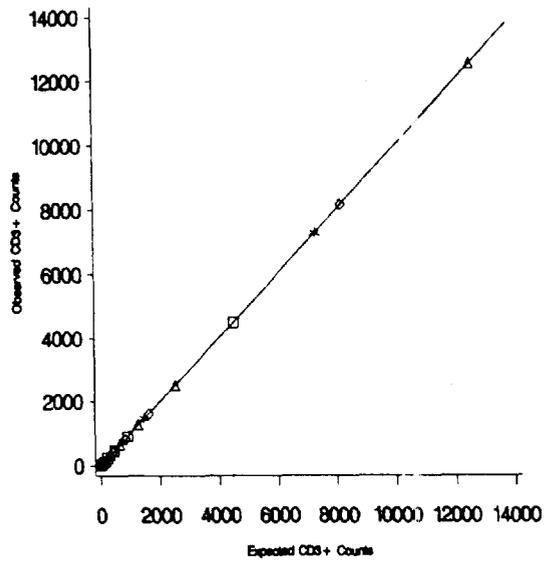
Linear regression analyses were performed as follows. The expected (X axis) values were calculated by multiplying the corresponding serial dilutions by the hematology analyzer derived buffy coat lymphocyte count and by the CYTORONABSOLUTE derived lymphocyte subset percent positive. The observed (Y axis) values were determined as the total lymphocyte count calculated from the hematology derived value of the concentrated sample times the CYTORONABSOLUTE derived lymphocyte subset percent positive at each dilution.

The OK-COMBO CD3/CD8 reagent demonstrated linear performance for both total CD3+ and CD3+CD8+ lymphocyte subsets across a lymphocyte count range of 20 cells/ul to 18,676 cells/ul as demonstrated with slopes indistinguishable from 1 and R values of 1.000.

Linear regression analyses of observed versus expected values for total percent CD3+ and percent CD3+CD8+ cells for each donor specimen are shown in Chart 5 and Chart 6 respectively. Regression analysis statistics are provided in Table 6.

CHART 5

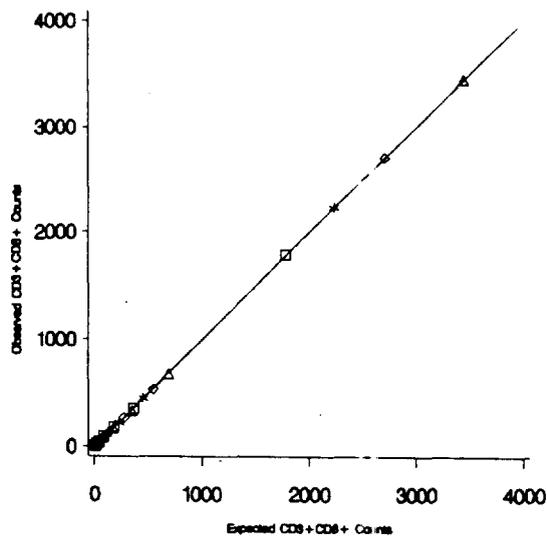
OK - COMBO CD3/CD8 CD3+



DONOR +--+ 71 □-□ 72 ◇-◇ 73 ▲-▲ 74

CHART 6

OK - COMBO CD3/CD8 CD3 + CD8 +



DONOR *-*-* 71 □-□-□ 72 ◇-◇-◇ 73 △-△-△ 74

TABLE 6

LINEAR REGRESSION ANALYSIS						
OK-COMBO CD3/FITC/CD8-PE						
N = 4						
OK-COMBO CD3/CD8	Donor	SLOPE	CI	INTERCEPT	CI	R
TOTAL CD3 ⁺	1	0.999	0.003	10.907	6.694	1.000
TOTAL CD3 ⁺	2	0.999	0.003	4.833	4.327	1.000
TOTAL CD3 ⁺	3	0.999	0.003	5.103	9.583	1.000
TOTAL CD3 ⁺	4	0.999	0.003	12.567	12.589	1.000
TOTAL CD3 ⁺	All	0.999	0.001	7.870	3.552	1.000
CD3 ⁺ CD8 ⁺	1	1.000	0.003	-0.889	2.414	1.000
CD3 ⁺ CD8 ⁺	2	0.999	0.005	-0.144	3.352	1.000
CD3 ⁺ CD8 ⁺	3	0.999	0.005	-0.657	5.106	1.000
CD3 ⁺ CD8 ⁺	4	1.001	0.005	-5.762	6.280	1.000
CD3 ⁺ CD8 ⁺	All	1.000	0.002	-1.868	1.935	1.000

CONCLUSION

Based upon the linear regression analysis of the clinical study data, and the supplemental studies performed demonstrating equivalence of the CYTORONABSOLUTE flow cytometer to the recognized flow cytometers, Becton Dickinson FACScan, and COULTER EPICS, Ortho-mune OK-COMBO CD3-FITC/CD8-PE (OKT3/OKT8) Monoclonal Antibody (Murine) is equivalent to the single color immunophenotyping reagents OKT3 Monoclonal Antibody (Murine) FITC Conjugate and Ortho-mune OKT8 Monoclonal Antibody (Murine) Phycoerythrin Conjugate for identification and determination of percent CD3⁺ and CD8⁺ lymphocytes in whole blood by flow cytometry.