

K 951632

MAR - 6 1997

ITEM 3

ATTACHMENT I

510(k) SUMMARY

FOR

**ORTHO-MUNE™ OK-COMBO CONTROL IgG_{2a}-FITC/IgG_{2a}-PE
MONOCLONAL ANTIBODY (MURINE)**

510(k) SUMMARY

SUBMITTER: Ortho Diagnostic Systems Inc.
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DEVICE NAME:

Ortho-mune™ OK-COMBO Control
IgG_{2a}-FITC/IgG_{2a}-PE
Monoclonal Antibody (Murine)

PREDICATE:

Simultest™ IMK Plus
Reagent B-Control
(K900078)

DATE: December 19, 1996

DEVICE DESCRIPTION:

Ortho-mune OK-COMBO Control IgG_{2a}-FITC/IgG_{2a}-PE Monoclonal Antibody (Murine) is a blend of murine monoclonal antibodies, not specific for human cellular antigens, conjugated to the fluorochromes, fluorescein isothiocyanate (FITC) and phycoerythrin (PE), respectively.

INTENDED USE:

Ortho-mune OK-COMBO Control is intended for use as negative control for immunophenotyping of human lymphocytes in whole blood by flow cytometry using Ortho-mune OK-COMBO immunophenotyping reagents. Ortho-mune OK-COMBO immunophenotyping reagents are blends of two purified monoclonal antibodies conjugated to fluorescein isothiocyanate and phycoerythrin, respectively. The negative control is used to check for nonspecific background staining and to set the negative/positive regions of the fluorescent cytograms.

TECHNOLOGICAL CHARACTERISTICS:

Both Ortho-mune OK-COMBO Control and Simultest IMK Plus Reagent B-Control use monoclonal antibodies which are not specific for human cellular antigens, purified and conjugated to fluorescein isothiocyanate and phycoerythrin.

PERFORMANCE DATA:

Performance of Ortho-mune OK-COMBO Control was compared to Simultest IMK Plus Reagent B-Control at three external, geographically distinct sites.

Whole blood specimens from 203 normal donors and 84 AIDS/ARC patients were stained and analyzed using the ORTHO CYTORONABSOLUTE™ laser flow cytometer (Ortho Diagnostic Systems Inc.).

The percent of events that fell within the positive region were analyzed to determine the expected mean, standard deviation (SD) and range for Ortho-mune OK-COMBO Control and Simultest IMK Control. These data are shown in Table A (Normal Donor Samples) and Table B (AIDS/ARC Samples).

TABLE A								
PERCENT OF EVENTS WITHIN THE POSITIVE REGION								
EXPECTED VALUES								
NORMAL DONOR SAMPLES N = 203								
Fluoro- chrome	Ortho-mune OK-COMBO Control			Simultest IMK Control			Comparison	
	Mean % in +Region	2SD	Range*	Mean % in +Region	SD	Range*	ΔMean	CI
FITC	0.32	0.50	0 - 1.32	0.25	0.16	0 - 0.56	0.07	0.07
PE	0.38	0.78	0 - 1.95	0.25	0.29	0 - 0.84	0.13	0.12

TABLE B								
PERCENT OF EVENTS WITHIN THE POSITIVE REGION								
EXPECTED VALUES								
AIDS/ARC SAMPLES N = 84								
Fluoro- chrome	Ortho-mune OK-COMBO Control			Simultest IMK Control			Comparison	
	Mean % in +Region	2SD	Range*	Mean % in +Region	SD	Range*	ΔMean	CI
FITC	0.43	0.34	0 - 1.10	0.31	0.20	0 - 0.71	0.11	0.08
PE	0.44	0.56	0 - 1.55	0.31	0.14	0 - 0.59	0.13	0.12

*Calculated

Reproducibility studies were performed at three independent laboratories using samples with low, normal and high percent positive lymphocyte subsets. The samples were processed to produce CD4 depleted, CD8 depleted, CD19 depleted and normal sample types. A portion of each sample was stained in replicates of ten with Ortho-mune OK-COMBO Control and analyzed using the ORTHO CYTORONABSOLUTE laser flow cytometer.

The percent of events within the negative region was calculated on all four sample types. 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 of the average 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 are provided in Table C.

TABLE C							
WITHIN LABORATORY REPRODUCIBILITY							
Ortho-mune OK-COMBO Control							
Percent of Events in the Negative Region							
N = 11 donors							
Sample Type	All SITES Mean Percent in Negative Region with CI*	Site A		Site B		Site C	
		CV	# Reps	CV	# Reps	CV	# Reps
CD19 Depleted	99.318 +/- 0.027	0.403	110	0.096	110	0.085	110
CD4 Depleted	99.314 +/- 0.057	0.151	99	0.860	110	0.079	109
CD8 Depleted	99.332 +/- 0.009	0.084	110	0.073	110	0.080	106
Normal	99.306 +/- 0.015	0.215	110	0.078	110	0.080	110

*Confidence Interval (CI) is at 95%.

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 are provided in Table D.

TABLE D					
BETWEEN LABORATORY REPRODUCIBILITY					
Ortho-mune OK-COMBO Control					
Percent of Events in the Negative Region					
N = 11 donors					
Sample Type	SITE A	SITE B	SITE C	ACROSS SITE	
	Mean Percent in Negative Region with CI* (All Donors)	Mean Percent in Negative Region with CI* (All Donors)	Mean Percent in Negative Region with CI* (All Donors)	Coefficient of Variation	# Rep
CD19 Depleted	99.304 +/- 0.403	99.345 +/- 0.096	99.305 +/- 0.085	0.024	330
CD4 Depleted	99.324 +/- 0.151	99.262 +/- 0.860	99.363 +/- 0.079	0.052	318
CD8 Depleted	99.358 +/- 0.084	99.334 +/- 0.073	99.303 +/- 0.080	0.028	326
Normal	99.311 +/- 0.215	99.326 +/- 0.077	99.281 +/- 0.080	0.023	330

*Confidence Interval (CI) is at 95%.

Ortho-mune OK-COMBO Control demonstrates acceptable within and between laboratory reproducibility for all sample types (CD4 depleted, CD8 depleted, CD19 depleted and normal).

A linearity study was performed using automated hematology analyzers and the ORTHO CYTORONABSOLUTE laser flow cytometer to determine the white blood cell count and percent lymphocytes of the normal donor samples being used in this study.

Four normal donor samples (whole blood, EDTA) were processed to produce samples with low, normal and high numbers of lymphocyte subsets. Each whole blood sample was concentrated by harvesting the buffy coat to obtain a white blood cell (WBC) count between 20,000 to 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 immunophenotyping reagents and analyzed using the ORTHO CYTORONABSOLUTE laser flow cytometer.

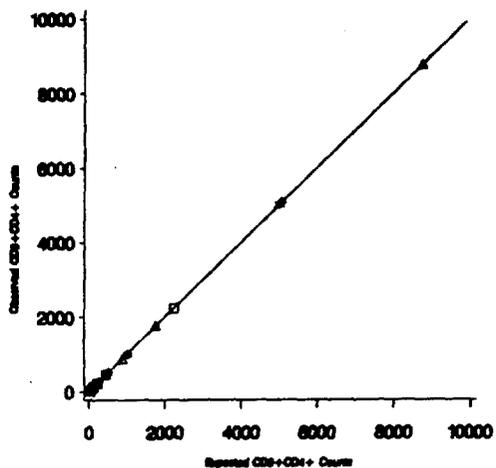
Least square linear regression analyses were performed as follows. The lymphocyte count for the buffy coat was derived by multiplying the WBC results from the hematology analyzer times the percent lymphocyte from the hematology analyzer. The expected (X axis) values were calculated by multiplying the corresponding serial dilutions by the hematology analyzer derived buffy coat lymphocyte count and by the ORTHO CYTORONABSOLUTE derived lymphocyte subset percent positive at each dilution.

Linearity results for two specificities of clinical significant, CD4 and CD19, are provided in Tables E and F, and Charts A and B to illustrate that Ortho-mune OK-COMBO Control can be used to establish analysis regions over a broad range of lymphocyte counts. Linearity graphs for each donor (donor #71 - donor #74) are presented for OK-COMBO CD3/CD4 (CD4 percent positive) in Chart A and for OK-COMBO CD3/CD19 (CD19 percent positive) in Chart B. The slope with confidence interval, intercept with confidence interval, and correlation coefficients are provided for OK-COMBO CD3/CD4 (CD4 percent positive) and OK-COMBO CD3/CD19 (CD19 percent positive) in Tables E and F.

TABLE E						
LINEARITY						
OK-COMBO CD3/CD4						
N = 4						
OK-COMBO CD3/CD4	Donor	SLOPE	CI	INTERCEPT	CI	R
CD3 ⁺ CD4 ⁺	71	0.999	0.003	7.735	5.402	1.000
CD3 ⁺ CD4 ⁺	72	1.000	0.003	1.512	2.233	1.000
CD3 ⁺ CD4 ⁺	73	0.999	0.004	4.855	7.155	1.000
CD3 ⁺ CD4 ⁺	74	0.999	0.003	13.555	10.706	1.000
CD3 ⁺ CD4 ⁺	All	0.999	0.002	6.516	3.118	1.000

TABLE F						
LINEARITY						
OK-COMBO CD3/CD19						
N = 4						
OK-COMBO CD3/CD19	Donor	SLOPE	CI	INTERCEPT	CI	R
TOTAL CD19 ⁺	71	1.004	0.009	-2.895	3.922	1.000
TOTAL CD19 ⁺	72	1.003	0.013	-1.702	4.146	1.000
TOTAL CD19 ⁺	73	1.002	0.004	-2.003	1.730	1.000
TOTAL CD19 ⁺	74	1.007	0.018	-18.749	14.665	1.000
TOTAL CD19 ⁺	All	1.003	0.008	-5.916	4.016	1.000

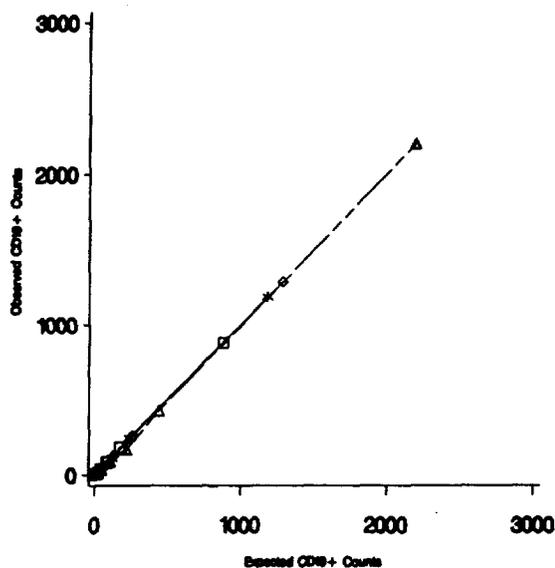
OK - COMBO CD3/CD4
CD3+CD4+



DONOR ◀-▶-▶ 71 □-□-□ 72 ◆-◆-◆ 73 ☆-☆-☆ 74

CHART A

OK-COMBO CD3/CD19
CD19+



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

CHART B

Performance of Ortho-mune OK-COMBO Control when used with Ortho-mune OK-COMBO immunophenotyping reagents was compared with that of the Becton Dickinson (BD) Simultest IMK Plus Reagent B-Control when used with Simultest immunophenotyping reagents. Data from studies comparing CD3/CD19 and CD4CD8 reagents are presented below.

Performance of the two color reagents Ortho-mune OK-COMBO CD3-FITC/CD19-PE (OKT3/OKB19A) Monoclonal Antibody (Murine) and Ortho-mune OK-COMBO CD4-FITC/CD8-PE (OKT4A/OKT8) Monoclonal Antibody (Murine) using Ortho-mune OK-COMBO Control was compared with that of Simultest IMK Plus Reagent C-Anti-Leu-4/Anti-Leu-12 (CD3/CD19) and Simultest IMK Plus Reagent-D-Anti-Leu3a/Anti-Leu-2a (CD4/CD8) using Simultest IMK Plus Reagent B-Control. Whole blood specimens from normal donors and AIDS/ARC patients were stained and analyzed using the ORTHO CYTORONABSOLUTE laser flow cytometer.

For each specimen, the percentage of gated cells which showed positive staining by each marker was calculated. The mean and range of the percent of positive stained cells for the normal donor population are shown in Tables G and H, and for the AIDS/ARC population in Tables I and J. The Ortho-mune OK-COMBO reagents and the Simultest IMK Plus reagents gave equivalent results for the CD3, CD19, CD4, and CD8 markers when used to stain whole blood from normal donors and AIDS/ARC patients.

TABLE G					
PERCENT POSITIVE STAINED CELLS IN NORMAL DONORS DETECTED BY OKT3/OKB19A AND LEU4/LEU12					
N = 191					
Ortho-mune Reagent	Mean %	Range %	BD Reagent	Mean %	Range %
OKT3	75.0	56.4-88.6	LEU4	73.9	53.6-88.5
OKB19A	14.0	1.4-32.1	LEU12	13.7	2.2-30.9

TABLE H					
PERCENT POSITIVE STAINED CELLS IN NORMAL DONORS DETECTED BY OKT4A/OKT8 AND LEU3a/LEU2a					
N = 190					
Ortho-mune Reagent	Mean %	Range %	BD Reagent	Mean %	Range %
OKT4A	47.5	11.6-70.8	LEU3a	44.1	13.1-61.2
OKT8	27.8	10.9-72.8	LEU2a	29.5	12.5-69.1

TABLE I					
PERCENT POSITIVE STAINED CELLS IN AIDS/ARC PATIENTS DETECTED BY OKT3/OKB19A AND LEU4/LEU12					
N = 85					
Ortho-Mune Reagent	Mean %	Range %	BD Reagent	Mean %	Range %
OKT3	76.9	39.4-93.6	LEU4	76.1	40.8-92.2
OKB19A	8.9	0.5-41.8	LEU12	9.9	0.3-41.1

TABLE J					
PERCENT POSITIVE STAINED CELLS IN AIDS/ARC PATIENTS DETECTED BY OKT4A/OKT8 AND LEU3a/LEU2a					
N = 83					
Ortho-mune Reagent	Mean %	Range %	BD Reagent	Mean %	Range %
OKT4A	18.2	0.1-60.4	LEU3a	15.8	0.2-50.4
OKT8	56.1	12.6-81.9	LEU2a	58.9	22.5-88.4

Studies were also performed using the Becton Dickinson FACScan™ flow cytometer. Whole blood samples were collected from normal donors. These samples were tested as collected to provide normal range cell counts, and were also concentrated 5-fold and diluted 5-fold to yield high range and low range cell counts. Samples were stained with Ortho-mune OK-COMBO reagents and with the Simultest IMK Plus reagents of comparable specificity. Ortho-mune OK-COMBO Control and Simultest IMK Plus Reagent B-Control were used with their respective OK-COMBO and Simultest reagents. The samples stained with the OK-COMBO reagents were analyzed on both the ORTHO CYTORONABSOLUTE and FACScan flow cytometers. The same samples stained with Simultest reagents were analyzed on the FACScan flow cytometer.

The mean percentage and range of positive stained cells for each marker were calculated. The results with Ortho-mune OK-COMBO reagents analyzed on both the ORTHO CYTORONABSOLUTE and BD FACScan flow cytometers are provided in Table K and Table L. The results comparing the OK-COMBO reagents with the Simultest reagents analyzed on the FACScan flow cytometer are provided in Table M and Table N.

These data demonstrate equivalent performance of the Ortho-mune OK-COMBO reagents using OK-COMBO Control when analyzed on both the ORTHO CYTORONABSOLUTE and BD FACScan flow cytometers, and equivalent performance of the OK-COMBO reagents using OK-COMBO Control to the Simultest IMK Plus reagents using Simultest IMK Plus Reagent B-Control when analyzed on the FACScan flow cytometer.

TABLE K				
OKT3/OKB19A ASSAYED ON FACSCAN AND CYTORONABSOLUTE				
N = 29				
Ortho-mune Reagent	FACScan		CYTORONABSOLUTE	
	Mean %	Range %	Mean %	Range %
OKT3	68.9	52.0-90.0	68.8	51.1-83.5
OKB19A	12.1	4.0-26.0	11.7	4.2-25.4

TABLE L				
OKT4A/OKT8 ASSAYED ON FACSCAN AND CYTORONABSOLUTE				
N=49				
Ortho-mune Reagent	FACScan		CYTORONABSOLUTE	
	Mean %	Range %	Mean %	Range %
OKT4A	51.5	35.0-68.0	48.9	32.3-63.7
OKT8	26.7	18.0-39.0	24.8	14.0-39.3

TABLE M					
OKT3/OKB19A Vs. LEU4/LEU12 ASSAYED ON THE FACSCAN					
N = 29					
Ortho-mune Reagent	FACScan		BD Reagent	FACScan	
	Mean %	Range %		Mean %	Range %
OKT3	68.9	52.0-90.0	LEU4	69.9	50.0-85.0
OKB19A	12.1	4.0-26.0	LEU12	14.0	6.0-29.0

TABLE N					
OKT4A/OKT8 Vs. LEU3a/LEU2a ASSAYED ON THE FACSCAN					
N = 49					
Ortho-mune Reagent	FACScan		BD Reagent	FACScan	
	Mean %	Range %		Mean %	Range %
OKT4A	51.5	35.0-68.0	LEU3a	49.2	36.0-62.0
OKT8	26.7	18.0-39.0	LEU2a	29.1	20.0-40.0

In addition, the performance of the following Ortho-mune OK-COMBO immunophenotyping reagents when used with Ortho-mune OK-COMBO Control has been compared to the performance of various other single color and two color reagents and Simultest CD3/CD4 (Leu-4/3a [K913912] in the following 510(k) Premarket Notifications.

Reagent	K#	Date Cleared
Ortho-mune OK - COMBO CD3-FITC/CD4-PE (OKT3/OKT4A)	K950568	May 13, 1996
Ortho-mune OK - COMBO CD3-FITC/CD8-PE (OKT3/OKT8)	K950482	May 10, 1996
Ortho-mune OK - COMBO CD4-FITC/CD8-PE (OKT4A/OKT8)	K950625	May 13, 1996
Ortho-mune OK-COMBO CD3-FITC/CD19-PE (OKT3/OKB19A)	K951100	May 13, 1996

CONCLUSION:

Performance of Ortho-mune OK-COMBO Control IgG_{2a}-FITC/IgG_{2a}-PE Monoclonal Antibody (Murine) is substantially equivalent to Simultest IMK Plus Reagent B-Control when used with Ortho-mune OK-COMBO and Simultest immunophenotyping reagents, respectively.

Ortho-mune OK-COMBO Control IgG_{2a}-FITC/IgG_{2a}-PE Monoclonal Antibody (Murine) when used with other Ortho-mune OK-COMBO immunophenotyping reagents is substantially equivalent to various other single and two color immunophenotyping reagents when used with their respective controls.