CALTAG
LABORATORIES

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510(K) SUMMARY
SUMMARY OF SAFETY AND EFFECTIVENESS DATA

CD3 FITC/CD19 R-PE/CD45 TRI-COLOR™
Mouse Monoclonal Antibody Combination
To Human Cell Surface Antigens by Flow Cytometry

NAME AND LOCATION OF MANUFACTURER:

Caltag Laboratories, Inc.
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Burlingame, CA 94010
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NAME OF CONTACT PERSON:

Robert C. Johnson
Executive Vice President
Caltag Laboratories, Inc.

DATE OF PREPARATION OF SUMMARY:

December 1, 1996

TRADE NAME OF THE DEVICE:

CD3 FITC/CD19 R-PE/CD45 TRI-COLOR™
Mouse Monoclonal Antibody Combination
To Human Cell Surface Antigens by Flow Cytometry

COMMON NAME:

Caltag CD3 FITC/CD19 R-PE/CD45 TRI-COLOR™ Monoclonal Antibody Combination

CLASSIFICATION NAME:

Automated Differential Cell Coulter (21 CFR 864.5220)

LEGALLY MARKETED DEVICE (PREDICATE DEVICE) TO WHICH THE MANUFACTURER IS CLAIMING SUBSTANTIAL EQUIVALENCE:

The Caltag CD3 FITC monoclonal antibody in the CD3 FITC/CD19 R-PE/CD45 TRI-COLOR combination is substantially equivalent to the Caltag CD3 FITC single monoclonal antibody for in vitro diagnostic use.

The Caltag CD19 R-PE monoclonal antibody in the CD3 FITC/CD19 R-PE/CD45 TRI-COLOR combination is substantially equivalent to the Caltag CD19 R-PE single monoclonal antibody for in vitro diagnostic use, and is substantially equivalent to the Coulter CD19 RD1 single monoclonal antibody for in vitro diagnostic use.

DESCRIPTION OF THE DEVICE:

The CALTAG CD3/CD19/CD45 monoclonal antibody combination binds to the surfaces of viable blood cells that express the corresponding antigens. To identify cells bearing these antigenic determinants, peripheral blood leukocytes are incubated with the monoclonal antibody, and washed to remove unbound antibody. Prior to removal of unbound antibody, lysis solution is added to lyse red blood cells. An appropriate fixative solution is added to lysed and washed cells. Stained and fixed cells are subsequently analyzed by flow cytometric methods.

INTENDED USE OF THE DEVICE:

CALTAG CD3/CD19/CD45 is a fluorescent reagent containing a combination of CD3, CD19 and CD45 monoclonal antibodies conjugated to fluorescein, phycoerythrin and the tandem fluorochrome PE-Cy5, respectively. This reagent permits the simultaneous identification of CD3+ mature T lymphocytes, CD19+ mature B lymphocytes, and CD45+ leukocytes including lymphocytes, monocytes and granulocytes, by flow cytometric methods.

SUMMARY OF THE TECHNICAL CHARACTERISTICS OF THE MANUFACTURER'S DEVICE COMPARED TO THE PREDICATE DEVICE:

Comparisons of Caltag Monoclonal Antibody Components to Caltag and Coulter Single Monoclonal Antibodies

No.	Item	Caltag Components	Caltag Antibodies Coulter Antibody	Comparison
1.	Intended Use	Flow Cytometry	Flow Cytometry Immunofluorescence	Substantially equivalent
2.	Specificity	CD3 CD19 CD19 CD45	CD3 Caltag CD19 Caltag CD19 Coulter	Substantially equivalent "live gating" only
3.	Target cell for CD3	Mature T Lymphocyte	Mature T Lymphocytes	Substantially equivalent
	for CD19	B lymphocytes	B lymphocytes	Substantially equivalent
	for CD45	All Leukocytes	All Leukocytes	"live gating" only
4.	Chemical form	Monoclonal antibody	Monoclonal antibody	Substantially equivalent
5.	Fluorochromes	FITC R-PE R-PE TRI-COLOR	FITC R-PE (Caltag) RD1 (Coulter) not available (Coulter)	Substantially equivalent
6.	Available forms FITC PE TRI-COLOR	 liquid, PBS liquid, PBS liquid, PBS	 lyophilized liquid, PBS none available (Coulter)	 Substantially equivalent
7.	Sample prep. methods	whole blood	whole blood	Substantially equivalent

NON CLINICAL TESTS SUPPORTING A DETERMINATION OF SUBSTANTIAL EQUIVALENCE:

EXPECTED VALUE DATA

Blood samples were collected from a total of 155 apparently healthy normal donors in an age range of 16 to 72, with a mean age of 41, for the determination of expected values of the Caltag CD3/CD19/CD45 monoclonal antibody combination. Samples were collected and analyzed in each of three independent laboratories. An approximately equal number of males and females were collected and analyzed in each laboratory.

The population contained members of differing ethnic origins, including adult Caucasians, Blacks, Orientals and Hispanics. Donors in geographically diverse areas of the United States, including the Eastern, SouthCentral and Western regions, participated in this study.

Summary of expected values for CALTAG CD3 FITC CD19 R-PE and CD45 TRI-COLOR components for 155 normal donors:

procedure	mean % positive	S.D.	Range ±2 S.D.
CD3 FITC component	72.8	7.7	57-88
CD19 R-PE component	13.3	4.5	4-22
CD45 TRI-COLOR component	100.0	0.1	100-100

Comparison of CALTAG CD3 FITC, CD19 R-PE and CD45 TRI-COLOR components to the CALTAG CD3 FITC, CD19 R-PE and CD45 TRI-COLOR single antibodies. Blood samples were collected from different randomly selected populations of adult normal donors having similar age, gender and ethnic distributions for the determination of expected values for the Caltag CD3 FITC and CD45 TRI-COLOR single monoclonal antibodies, and from the same population of adult normal donors for the determination of expected values for Caltag CD19 R-PE single antibody.

SINGLE ANTIBODIES

procedure	mean % positive	S.D.	Range ±2 S.D.	n
CD3 FITC	71.8	6.9	58-86	130
CD19 R-PE	13.0	4.2	5-21	155
CD45 TRI-COLOR	99.0	0.9	97-100	40

Expected values for pediatrics and adolescents have not been determined.

The values obtained from normal individuals may vary from laboratory to laboratory; therefore, it is recommended that each laboratory establish its own normal range.

SPECIFICITY DATA

Blood samples were obtained from healthy normal donors of Caucasian, Black, Hispanic and Oriental ethnic origins. Samples of each donor were stained with CALTAG CD3 FITC/CD19 R-PE/CD45 TRI-COLOR monoclonal antibody combination. Cells contained in the lymphocyte, monocyte and granulocyte regions were selected for analysis. Separate samples from the same donors were prepared for analysis of red blood cells and platelets and stained with each of the CALTAG monoclonal antibodies.

CD3 FITC Component

Ethnic Origin	Percent of Stained Cells				
	Lymph.	Mono.	Gran.	Plt.	RBC
Caucasian	65.2	1.7	1.5	0.6	0.3
Caucasian	81.4	1.4	0.5	0.3	0.4
Hispanic	79.2	1.9	0.6	0.3	0.4
Oriental	81.2	1.3	0.9	0.4	0.2
Black	84.9	0.9	0.6	0.4	0.4
Mean	78.4	1.4	0.8	0.4	0.3
±1 S.D.	7.6	0.4	0.4	0.1	0.1

CD19 R-PE Component

Ethnic Origin	Percent of Stained Cells				
	Lymph.	Mono.	Gran.	Plt.	RBC
Caucasian	18.1	1.3	0.5	0.4	0.2
Caucasian	12.9	0.6	0.4	0.2	0.2
Hispanic	13.4	0.5	0.6	0.2	0.6
Oriental	12.1	0.8	0.5	0.3	0.3
Black	12.7	0.9	0.3	0.4	0.2
Mean	13.8	0.8	0.5	0.3	0.3
±1 S.D.	2.4	0.3	0.1	0.1	0.2

CD45 TRI-COLOR Component

Ethnic Origin	Percent of Stained Cells				
	Lymph.	Mono.	Gran.	Plt.	RBC
Caucasian	100.0	100.0	100.0	0.8	0.2
Caucasian	100.0	100.0	100.0	0.5	0.3
Hispanic	100.0	100.0	100.0	0.3	0.4
Oriental	100.0	100.0	100.0	0.4	0.3
Black	100.0	100.0	100.0	0.3	0.2
Mean	100.0	100.0	100.0	0.5	0.3
±1 S.D.	0.0	0.0	0.0	0.2	0.1

Specific and/or nonspecific antibody Fc binding to monocytes in a patient sample can be excluded by proper gating on lymphocytes on the flow cytometer.

CLINICAL TESTS SUPPORTING A DETERMINATION OF SUBSTANTIAL EQUIVALENCE:

CORRELATION DATA

The Correlation study was performed on 175 donors, including 155 normal and 20 abnormal donors.

Comparison of the CALTAG CD3 FITC conjugated monoclonal antibody component of CD3 FITC/CD19 R-PE/CD45 TRI-COLOR with the CALTAG CD3 FITC conjugated single monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept
CD3 FITC component	69.7	94.8	1.01	0.33
CD3 FITC single	68.3			

CD3 FITC component
Slope + 1.01
y intercept + 0.33
Linear regression $y = 0.33 + 1.01x$

Comparison of the CALTAG CD19 R-PE conjugated monoclonal antibody component of CD3 FITC/CD19 R-PE/CD45 TRI-COLOR with the Caltag CD19 R-PE conjugated single monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept
CD19 R-PE component	16.8	98.9	1.02	0.08
CD19 R-PE single	16.4			

CD19 R-PE component
Slope + 1.02
y intercept + 0.08
Linear regression $y = 0.08 + 1.02x$

Comparison of the CALTAG CD19 R-PE conjugated monoclonal antibody component of CD3 FITC/CD19 R-PE/CD45 TRI-COLOR with the Coulter CD19 RD1 conjugated single monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept
CD19 R-PE component	16.8	98.1	0.95	1.29
Coulter CD19 RD1	16.2			

CD19 R-PE component
Slope + 0.95
y intercept + 1.29
Linear regression $y = 1.29 + 0.95x$

BIBLIOGRAPHY

1. Hsu S., Cossman J., Jaffe E.; Lymphocyte subsets in normal human lymphoid tissues. Am. J. Clin. Path. 80:21-30, 1983.
2. Morimoto C., Letvin N.L., Distaso J.A., et al: The cellular basis for the induction of antigen-specific T8-suppressor cells. Eur. J. Immunol. 16:198-204, 1986.
3. Kurrle R., Cluster Report: CD3, in Fourth International Workshop And Conference On Human Leukocyte Differentiation Antigens, pp 290-293, Vienna, 1989.
4. Transy C., Moingeon P.E., Marshall B., et al: Most murine anti-human CD3 mAb recognize the human CD3 epsilon subunit. in Fourth International Leukocyte Workshop and Conference On Human Leukocyte Differentiation Antigens. pp 293-295, Vienna, 1989.
5. Dorken B., Moller P., Pezzutto A., Schwartz-Albiez R., Moldenhauer G., B-cell antigens:CD19, in Fourth International Workshop and Conference On Human Leucocyte Differentiation Antigens, pp 34-36, Vienna, 1989.

6. Pirruccello S.J., Johnson D.R., Reagents for flow cytometry: Monoclonal antibodies and hematopoietic cell antigens, in Flow Cytometry And Clinical Diagnosis, pp 56-78, pub. American Society of Clinical Pathologists, 1994.
7. Shields J.G., Rigley K.P., Callard R.E., Regulation of human B cell proliferation by the CD19 cell-surface glycoprotein, in Fourth International Workshop and Conference On Human Leucocyte Differentiation Antigens, pp 40-43, Vienna, 1989.
8. Schwinzer R., Cluster Report CD45/CD45R, in Fourth International Workshop and Conference On Human Leucocyte Differentiation Antigens, pp 628-634, Vienna, 1989.
9. Fifth International Workshop and Conference On Human Leucocyte Differentiation Antigens, Leucocyte Typing V, White Cell Differentiation Antigens, Boston, 1993.
10. Reinherz E.L., Cooper M.D., Schlossman S.F., Abnormalities of T cell maturation and regulation in human beings with immunodeficiency disorders. J. Clin. Invest. 68:609-705, 1981.
11. Schmidt R.E., Monoclonal antibodies for diagnosis of immunodeficiencies. Blut 59:200-206, 1989.
12. Smolen J.S., Morimoto C., Steinberg A.D., et al: Systemic lupus erythematosus: delineation of subpopulations by clinical serologic and T cell marker analysis. Am. J. Med. Sci. 289:139-141, 1985.
13. Morimoto C., Steinberg A.D., Letvin A.L., et al: A defect of immunoregulatory T cell subsets in SLE patients demonstrated with anti-2H4. J. Clin. Invest. 79:762-765, 1987.
14. Duke O., Panayi G.S., Janossy G., et al: Analysis of T cell subsets in the peripheral blood and synovial fluid of patients with rheumatoid arthritis by means of monoclonal antibodies. Ann Rheum Dis. 42:357-364, 1983.
15. Reinherz E.L., O'Brien C., Rosenthal P., et al: The cellular basis for viral-induced immunodeficiency: Analysis by monoclonal antibodies. J. Immunol. 125:1266-1274, 1980.
16. Nadler L.M., in Leukocyte Typing II, Volume 2, B cell/Leukemia Panel Workshop: Summary and Comments, Chapter 1:2-20, Springer Verlag, New York, 1986.
17.. Wedgwood R.J., X-linked agammaglobulinemia, in CRC Handbook Series In Clinical Laboratory Science, CRC Press, West Palm Beach Florida, pp 41-50, 1978.
18. Pearl E.R., Vogler L.B., Okos A.J. et al, B lymphocyte precursors in bone marrow. An analysis of normal individuals and patients with antibody-deficiency states, J. Immunol. 120:1169-175, 1978.
19. Spickett G.P., Webster A.D., Farrant J., Cellular abnormalities in common variable immunodeficiency, Immunodefic. Rev. 2:199-219, 1990.
20. Small T.N., Keever C., Collins N. et al, Characterization of B cells in severe combined immunodeficiency disease, Hum. Immunol. 25:181-193, 1991.
21. Goldstein R., Izaguirre L.C., Douglas J. et al, Systemic lupus erythematosus and common variable panhypogammaglobulinemia. Theoretical and practical considerations, Fed. Proc. 29:1606-1611, 1985.
22. Schwinzer R., Cluster Report CD45/CD45R, in Fourth International Workshop and Conference On Human Leucocyte Differentiation Antigens, pp 628-634, Vienna, 1989.
23. Loken M.L., Brosnan N.N., Back, B.A., Ault K.A.: Establishing optimal lymphocyte gates for immunophenotyping for flow cytometry, Cytometry 11:453-459, 1990.
24. Mandy F.F., Bergeron M., Recktenwald D., Izaguirre C.A., A simultaneous three color T cell subsets analysis with single laser flow cytometers using T cell gating protocol, J. Immunol. Methods 156:151-162, 1992.
25. Stelzer G.T., Schults K.S., Loken M.R., CD45 gating for routine flow cytometric analysis of human bone marrow specimens, in Clinical Flow Cytometry, A. Landay, K. Ault, K. Bauer, P. Rabinovitch, eds, pp 265-280, 1993.
26. Stewart C.C., Stewart S.J., Multiparameter analysis of leukocytes by flow cytometry, in Methods Of Cell Biology, Z. Darzynkiewicz, H. Crissman eds, in press, 1994.
27. 1994 Revised Guidelines for the Performance of CD4+ T-Cell Determinations in Persons with Human Immunodeficiency Virus Infection, Morbidity And Mortality Weekly Report (MMWR), Volume 43/No. RR-3, March 4, 1994.
28. Nicholson J.K.A., Green T.A. and Collaborative Laboratories, Selection of anticoagulants for lymphocyte immunophenotyping. J. Immunol. Methods 165:31-35, 1993 .
29. Tennant J.R., Evaluation of the Trypan Blue technique for determination of cell viability, Transplantation 2:685-694, 1964.
30. Koepke J.A., Landay A.L.: Precision and Accuracy of Absolute Lymphocyte Counts, Clin. Immunol. and Immunopath. 52:19-27, 1989.
31. Brown M.C., Hoffman R.A., Kirchanski S., Controls for flow cytometers in hematology and cellular immunology, Ann. N.Y. Acad. Sci. 468:93-103, 1986.
32. Durrand R.E., Calibration of flow cytometer detector systems, Cytometry 2:192-193, 1981.