CALTAG
LABORATORIES

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510(K) SUMMARY
SUMMARY OF SAFETY AND EFFECTIVENESS DATA

CD19 R-PE, CD19 TRI-COLOR Mouse Monoclonal Antibodies To Human Cell Surface Antigens by Flow Cytometry

NAME AND LOCATION OF MANUFACTURER:

Caltag Laboratories, Inc.
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NAME OF CONTACT PERSON:

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DATE OF PREPARATION OF SUMMARY:

October 1, 1996

TRADE NAME OF THE DEVICE:

Caltag CD19 R-PE, CD19 TRI-COLOR Mouse Monoclonal Antibodies To Human Cell Surface Antigens by Flow Cytometry

COMMON NAME:

Caltag CD19 R-PE, CD19 TRI-COLOR Monoclonal Antibody

CLASSIFICATION NAME:

Automated Differential Cell Coulter (21 CFR 864.5220)

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

Caltag CD19 R-PE Monoclonal Antibody to Human Cell Surface Antigens is substantially equivalent to the Coulter CD19 RD1 Monoclonal antibody for in-vitro diagnostic use.

Caltag CD19 TRI-COLOR Monoclonal Antibody to Human Cell Surface Antigens is substantially equivalent to the Coulter CD19 FITC and CD19 RD1 monoclonal antibodies for in-vitro diagnostic use.

DESCRIPTION OF THE DEVICE:

The CALTAG CD19 R-PE and CD19 TRI-COLOR monoclonal antibodies bind to the surfaces of viable blood cells that express the CD19 antigen. To identify cells bearing the CD19 determinant, 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 CD19 R-PE and CD19 TRI-COLOR are fluorochrome conjugated monoclonal antibody reagents that may be used to enumerate CD19+ lymphocytes in human peripheral blood by flow cytometric methods.

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

Comparisons of Caltag CD19 and Coulter CD19 Monoclonal Antibodies

No.	Item	Caltag Antibodies	Coulter Antibodies	Comparison
1.	Intended Use	Flow Cytometry	Flow Cytometry Immunofluorescence	Substantially equivalent
2.	Specificity	CD19	CD19	Substantially equivalent
3.	Target cell	B lymphocyte	B lymphocyte	Substantially equivalent
4.	Chemical form	Monoclonal antibody	Monoclonal antibody	Substantially equivalent
5.	Fluorochromes	R-PE, TRI-COLOR	FITC, RD1	Substantially equivalent
6.	Available forms			
	FITC	liquid, PBS	lyophilized	Substantially
	PE	liquid, PBS	liquid, PBS	equivalent
	TRI-COLOR	liquid, PBS	not available	
7.	Sample prep. methods	whole blood	whole blood	Substantially equivalent
8.	Expected values from this study (n=155)			
	R-PE	5-21%	4-21% (RD1)	Substantially
	TRI-COLOR	4-24%	3-23% (FITC)	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. 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 normal donor population included members of differing ethnic origins, including adult Caucasian, Black, Oriental and Hispanic.

Donors in geographically diverse areas of the United States, including the Western, Eastern and SouthCentral regions, participated in this study. Blood samples collected from each donor were stained with the CALTAG CD19 R-PE and CD19 TRI-COLOR monoclonal antibodies.

Summary of expected values for CALTAG CD19 monoclonal antibodies for all normal donors:

procedure	mean % positive	S.D. ±2 S.D.	Range	n
CD19 R-PE	13.0	4.2	5-21	155
CD19 TRI-COLOR	13.9	5.0	4-24	155

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 CD19 R-PE and CD19 TRI-COLOR monoclonal antibodies. 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.

CD19 R-PE

Ethnic Origin	Percent of Stained Cells Lymph.	Mono.	Gran.	Plt.	RBC
Caucasian	18.0	0.6	0.9	0.5	0.5
Caucasian	13.3	1.1	0.8	0.3	0.7
Hispanic	12.2	0.7	0.8	0.4	1.0
Oriental	11.2	1.6	1.3	0.4	0.5
Black	14.6	0.0	0.5	0.6	0.9
Mean	13.9	0.8	0.9	0.4	0.7
±1 S.D.	2.6	0.6	0.3	0.1	0.2

CD19 TRI-COLOR

Ethnic Origin	Percent of Stained Cells Lymph.	Mono.	Gran.	Plt.	RBC
Caucasian	18.3	0.0	1.0	0.3	0.4
Caucasian	12.1	0.2	1.1	0.4	0.1
Hispanic	11.6	0.2	0.3	0.2	0.7
Oriental	11.0	1.0	0.9	0.3	0.2
Black	12.0	0.7	0.9	0.4	0.2
Mean	13.0	0.4	0.8	0.3	0.3
±1 S.D.	3.0	0.4	0.3	0.1	0.2

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.

REPRODUCIBILITY DATA (INTRA-LAB)

Intra-lab reproducibility for the CALTAG CD19 R-PE and CD19 TRI-COLOR conjugated monoclonal antibodies was determined by performing 10 replicated determinations for each antibody in each of three ranges; high, medium and low. Thus, a total of 30 determinations were performed for each form of CD19. In this manner, reproducibility was demonstrated throughout the entire measuring range.

The 10 determinations for each range were performed by the staining, processing and analysis of 10 separate samples. Lymphocytes were selected for the analysis of percent cells stained in each of the three ranges.

To perform this study, anticoagulated blood was obtained from an abnormal donor expressing a high percentage of CD19+ cells. Mid range and low range samples were obtained by adding known CD19- cells in appropriate ratios, while maintaining approximately the same total cell concentrations for the three ranges.

The study was performed in each of three independent laboratories, in the manner that each laboratory obtained, stained and analyzed separate blood samples. The following data are representative:

procedure	Level	mean % positive	S.D.	% CV	n
CD19 R-PE	high	66.6	0.4	0.5	10
	mid	46.7	0.8	1.6	10
	low	14.9	0.6	4.3	10

procedure	Level	mean % positive	S.D.	% CV	n
CD19 TRI-COLOR	high	65.4	0.7	1.0	10
	mid	46.1	0.6	1.3	10
	low	15.5	0.4	2.7	10

REPRODUCIBILITY, (INTER-LAB)

Inter-lab reproducibility for the CALTAG CD19 R-PE and CD19 TRI-COLOR conjugated monoclonal antibodies was determined by performing 10 replicated determinations for each antibody in each of three ranges; high, medium and low. Thus, a total of 30 determinations were performed for each form of CD19. In this manner, reproducibility was demonstrated throughout the entire measuring range.

The 10 determinations for each range were performed by the staining, processing and analysis of 10 separate samples. Lymphocytes were selected for the analysis of percent cells stained in each of the three ranges.

The study was performed in each of three laboratories. All laboratories stained and analyzed blood samples from the same blood donors. Lysed unstained samples containing cells in the appropriate ranges were prepared by one of the participating laboratories for staining and analysis in each of the laboratories. The following data were obtained:

SITE 1

procedure	Level	mean % positive	S.D.	% CV	n
CD19 R-PE	high	84.7	4.4	5.2	10
	mid	70.4	1.2	1.8	10
	low	42.7	3.2	7.6	10

procedure	Level	mean % positive	S.D.	% CV	n
CD19 TRI-COLOR	high	86.7	1.0	1.2	10
	mid	70.9	1.3	1.9	10
	low	42.7	0.9	2.1	10

SITE 2

procedure	Level	mean % positive	S.D.	% CV	n
CD19 R-PE	high	83.9	1.5	1.7	10
	mid	71.1	2.8	3.9	10
	low	42.5	1.9	4.6	10

procedure	Level	mean % positive	S.D.	% CV	n
CD19 TRI-COLOR	high	85.3	2.2	2.6	11
	mid	65.7	3.1	4.8	9
	low	32.6	2.0	6.0	10

SITE 3

procedure	Level	mean % positive	S.D.	% CV	n
CD19 R-PE	high	87.5	0.6	0.7	10
	mid	69.9	0.8	1.1	10
	low	38.8	1.6	4.2	10

procedure	Level	mean % positive	S.D.	% CV	n
CD19 TRI-COLOR	high	85.3	0.9	1.0	10
	mid	66.1	1.0	1.5	10
	low	30.7	2.4	7.9	10

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 CD19 R-PE conjugated monoclonal antibody with the Coulter CD19 RD1 conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 R-PE	16.4	97.7	0.92	1.30	175
CD19 RD1	16.2				

CD19 R-PE
Linear regression $y = 1.30 + 0.92x$

Comparison of the CALTAG CD19 R-PE conjugated monoclonal antibody with the Coulter CD19 FITC conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 R-PE	16.4	96.3	0.93	0.69	175
CD19 FITC	16.7				

CD19 R-PE
Linear regression $y = 0.69 + 0.93x$

Comparison of the CALTAG CD19 TRI-COLOR conjugated monoclonal antibody with the Coulter CD19 RD1 conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 TRI-COLOR	17.1	96.7	0.92	2.14	175
CD19 RD1	16.2				

CD19 TRI-COLOR
Linear regression $y = 2.14 + 0.92x$

Comparison of the CALTAG CD19 TRI-COLOR conjugated monoclonal antibody with the Coulter CD19 FITC conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 TRI-COLOR	17.1	97.4	0.94	1.36	175
CD19 FITC	16.7				

CD19 TRI-COLOR
Linear regression $y = 1.36 + 0.94x$

Comparison of the CALTAG CD19 TRI-COLOR conjugated monoclonal antibody with the CALTAG CD19 R-PE conjugated monoclonal antibody:

procedure	mean % positive	r^2 value	slope	Y intercept	n
CD19 TRI-COLOR	17.1	97.6	0.99	-0.56	175
CD19 R-PE	16.4				

CD19 TRI-COLOR
Linear regression $y = -0.56 + 0.99x$

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. 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.
4. 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.
5. 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.
6. 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.
7. Wedgwood R.J., X-linked agammaglobulinemia, in CRC Handbook Series In Clinical Laboratory Science, CRC Press, West Palm Beach Florida, pp 41-50, 1978.
8. 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.
9. Spickett G.P., Webster A.D., Farrant J., Cellular abnormalities in common variable immunodeficiency,
Immunodefic. Rev. 2:199-219, 1990.
10. Small T.N., Keever C., Collins N. et al, Characterization of B cells in severe combined immunodeficiency disease,
Hum. Immunol. 25:181-193, 1991.
11. 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.
12. 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.
13. Guidelines for the Performance of CD4+ T-Cell Determinations in Persons with Human Immunodeficiency Virus Infection, Morbidity And Mortality Weekly Report (MMWR), Volume 41/No. RR-8, May 8, 1992.
14. Nicholson J.K.A., Green T.A. and Collaborative Laboratories, Selection of anticoagulants for lymphocyte immunophenotyping. J. Immunol. Methods 165:31-35, 1993 .
15. Tennant J.R., Evaluation of the Trypan Blue technique for determination of cell viability, Transplantation 2:685-694, 1964.
16. Koepke J.A., Landay A.L.: Precision and Accuracy of Absolute Lymphocyte Counts,
Clin. Immunol. and Immunopath. 52:19-27, 1989.
17. 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.
18. Durrand R.E., Calibration of flow cytometer detector systems, Cytometry 2:192-193, 1981.