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(54) **DETECTION OF HUMAN ANTIBODIES TO SQUALENE IN SERUM**

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(52) **U.S. Cl.** ..... **435/7.1; 435/7.2; 435/7.9; 435/7.92**

(58) **Field of Classification Search** ..... **435/7.1, 435/7.2, 7.91, 7.52**  
See application file for complete search history.

(56) **References Cited**

**U.S. PATENT DOCUMENTS**

5,210,039	A *	5/1993	Cummins et al.	436/17
5,294,537	A *	3/1994	Batt	435/7.32
5,419,904	A	5/1995	Irie	
5,419,921	A	5/1995	Molacek et al.	
5,709,879	A *	1/1998	Barchfeld et al.	424/450
6,166,050	A	12/2000	Lombardo et al.	
6,191,108	B1	2/2001	Rodkey et al.	
6,214,566	B1	4/2001	Asa et al.	

**FOREIGN PATENT DOCUMENTS**

WO	WO 95/00146	A1	1/1995
WO	WO 98/20137	A1	5/1998
WO	WO 01/87302	A1	11/2001

**OTHER PUBLICATIONS**

Harlow et al. "Antibodies a Laboratory Manual," Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1988, p. 141-155.1.\*  
Applicant's filed affidavit & declaration in parent case (U.S. Appl. No. 09/859,389).\*

Alving, C.R. et al., "Preparation and Use of Liposomes in Immunological Studies". In: G. Gregoriadis (Ed.) Liposome Technology, vol. 3 (Second Ed.), CRC Press, Inc. Boca Raton, p. 317-343, year 1993.  
Mayes, P.A., "Cholesterol Synthesis, Transport, & Excretion", *Harp-er's Biochemistry*, 24<sup>th</sup> Edition, Appleton & Lang, Stamford, 1996, p. 271-283.

Schuster, B. et al., "Production of Antibodies Against Phosphocholine, Phosphatidylcholine, Sphingomyelin, and Lipid A by Injection of Liposomes Containing Lipid A", *J. Immunol.*, 1979, vol. 122, No. 3, pp. 900-905.

Stollar, B.D. et al., "Cross-Reactions of Nucleic Acids With Monoclonal Antibodies to Phosphatidylinositol Phosphate and Cholesterol", *Mol. Immunol.*, 1989, vol. 26, No. 1, pp. 73-79.

Swartz, Jr. G.M. et al., "Antibodies to Cholesterol", *Proc. Natl. Acad. Sci. U.S.A.*, 1988, vol. 85, No. 6, pp. 1902-1906.

Wassef, N.M. et al., "Phosphate Binding Specificities of Monoclonal Antibodies Against Phosphoinositides in Liposomes", 1984, vol. 21, No. 10, pp. 863-868, *Book Molecular Immunology*.

Phillips, Christopher J., et al., "Antibodies to squalene in US Navy Persian Gulf War veterans with chronic multisymptom illness," *Vaccine*, 2009, p. 391-3926, vol. 27.

Blanchard, Melvin S., et al., "Chronic Multisymptom Illness Complex in Gulf War I Veterans 10 Years Later," *American Journal of Epidemiology*, 2006, p. 66-75, vol. 163, No. 1.

Bonhoeffer, Jan, et al., "Adverse events following immunization: perception and evidence," *Curr Opin Infect Dis*, 2007, p. 237-246, vol. 20.

Del Giudice, Giuseppe, et al., "Vaccines with the MF59 Adjuvant Do Not Stimulate Antibody Responses against Squalene," *Olin. Vaccine Immunol.*, Sep. 2006, p. 1010-1013, vol. 13, No. 9.

Diaz-Torne, C., et al., "Absence of Histologic Evidence of Synovitis in Patients With Gulf War Veterans' Illness With Joint Pain," *Arthritis & Rheumatism*, Oct. 15, 2007, p. 1316-1323, vol. 57, No. 7.

Ferguson, Eamonn, et al., "Theoretical accounts of Gulf War Syndrome: From environmental toxins to psychoneuroimmunology and neurodegeneration," *Behavioural Neurology*, 2001/2002, p. 133-147, vol. 13.

Fraser, Claire M., "A Genomics-Based Approach to Biodefence Preparedness," *Nature Reviews*, Jan. 2004, p. 23-33, vol. 5.

Fukasawa, Lucila O., "Adjuvant can improve protection induced by OMV vaccine against *Neisseria meningitidis* serogroups B/C in neonatal mice," *FEMS Immunology and Medical Microbiology*, 2004, p. 205-210, vol. 41.

Hannan, K. L., et al., et al., "Activation of the coagulation system in Gulf War Illness: a potential pathophysiologic link with chronic fatigue syndrome A laboratory approach to diagnosis," *Blood Coagulation and Fibrinolysis*, 2000, p. 673-678, vol. 11, No. 7.

Holm, Barbro C. et al., "Rats made congenic for Oia3 on chromosome 10 become susceptible to squalene-induced arthritis," *Human Molecular Genetics*, 2001, p. 565-575, vol. 10, No. 6.

(Continued)

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(57) **ABSTRACT**

The invention is a method and assay for detecting human squalene antibodies in sera or plasma using a blocking agent of -0.5% casein in a phosphate buffered saline (PBS) solution.

**1 Claim, 25 Drawing Sheets**