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(71) Applicant: MUREX CORPORATION [US/US]; Post Office Box 2003, Norcross, GA 30091 (US).

(72) Inventor: STARK, Fred, Richard ; 797 Northwest 12th Street, Ontario, OR 97914 (US).

(74) Agent: BERNSTEIN, Jason, A.; Murex Corporation, Post Office Box 2003, Norcross, GA 30091 (US).

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(54) Title: DIAGNOSTIC KIT FOR SEXUALLY TRANSMITTED DISEASES

(57) Abstract

A kit for the detection of all agents in a diagnostic specimen known to indicate a sexually transmitted disease comprising: (a) means containing a plurality of spaced apart reaction zones, and (b) a plurality of monoclonal antibody reagents for use with said means; there being at least one said monoclonal antibody reagent for each such agent capable of reacting in a detectable immunologic manner with said agent.

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DIAGNOSTIC KIT FOR SEXUALLY TRANSMITTED DISEASES

BACKGROUND OF THE INVENTION

The present invention is directed to a diagnostic kit with a plurality of monoclonal antibody reagents, each of which is capable of reacting in an immunologic manner with an infective agent of a sexually transmitted disease (STD), such as Neisseria, Treponema, Chlamydia, Herpes, Trichomonas, Candida, and/or Gardnerella vaginalis, AIDS or AIDS associated retrovirus, Mycoplasma, Giardia, Entamoeba, hepatitis, Salmonella, Shigella, Campylobacter, Mobiluncus, cytomegalovirus, Group B Streptococcus, Staphylococcus aureus, Pneumocystis carynii, Bacteriodes fragilis, Legionella pneumophila, Hemophilus ducreyi, and Calymmatobacterium granulomatis. The monoclonal antibody can be labeled with fluorescent, luminescent, enzymes, isotopes, ferromagnetic atoms, or particles, or the like labels.

"Sexually transmitted diseases" is a term used to describe a disease that is spread by sexual contact. In particular, Neisseria gonorrhoeae is the most familiar of all venereal diseases, as well as the use of several vaccines to give immunity to this disease. Its symptoms and manifestations are complex, including local genital and systematic effects. Both Chlamydia and Trichomonas are now recognized as frequent causes of genital infections of men and women, while Gardnerella (G. vaginalis) and Candida (C. albicans) have been shown to be the most common cause of malodorous vaginitis and yeast infections, respectively, in women. Herpes, represented by Herpes hominus, is also a sexually transmitted infection often characterized by recurrent painful blisters of the genital region. Treponema (T. pallidium) is the causative agent of syphilis.

AIDS, or acquired immunodeficiency syndrome, represented by the human T-lymphotrophic virus (HTLV and its species), is a retrovirus transmitted primarily through intimate sexual contact and recently shown to be transmittable through the blood, causes a fatal deficiency of the immune system. Mycoplasma (e.g., m. hominis and Ureaplasma urealyticum) is a genus of aerobic to facultatively anaerobic bacteria associated with vaginitis, pelvic inflammatory disease and involuntary inflexibility in both male and female. Current diagnosis depends on culturing specimens on appropriate media with subsequent identification of isolates. Giardia (Entamoeba histolytica) is an amebic parasite causing tropic or amebic dysentery in man in warmer parts of the world. Hepatitis (hepatitis) is a virus causing liver inflammation and is transmitted by dirty needles among drug abusers, in blood, sweat, tears, semen, and the like, as well as by male homosexuals by anal intercourse and in heterosexuals.

With societal increase of the incidence of anal intercourse, predominatly among homosexual partners but also among heterosexuals, clinicians have reported a corresponding increase in the frequency of infections not previously considered sexually transmittable. Among the common intestinal infections include Salmonella, Shigella, campylobacter, and the like. Other pathogens include Mobiluncus, cytomegalovirus ("CMV"), Group B Streptococcus, Staphylococcus aureus (the cause of "Toxic Shock Syndrome"), Pneumocystis caryrii, Bacteriodes fragilis, Legionella pneumophila, Hemophilus ducreyi, Calymmatobacterium granulomatis, and the like.

Present methods of detecting the presence of STDs are not only cumbersome and slow, but lack specificity. For instance, many of the venereal diseases are diagnosed by direct examination of clinical material, or cultures and gram stains, which are often insensitive. further, present diagnostic procedures for STD generally test only on an individual basis, thus precluding the possibility of detecting more than one causative agent for the manifested clinical symptoms. For example, gonorrhea will often mask the signs and symptoms of syphilis. Thus, unless the physician simultaneously tests for both diseases, the patient will only be treated for one infection.

Moreover, existing detection methods may present inadequate information for diagnosis where several disease are simultaneously present in a patient. A physician, relying on clinical presentation, might select one test for the suspected disease, prescribe treatment based on a positive result and miss the other diseases. An incomplete diagnosis may lead to incomplete recovery or even the worsening of health where the patient is treated with a drug that suppresses the body's ability to fight the other undiagnosed diseases. For example, a doctor might test a patient for Neisseria gonorrhoeae and fail to detect the presence of Chlamydia trachomatis, because the two have similar symptom presentations. Moreover, a female is often asymptomatic for a number of infections, which might be masked by the detection of only one infection.

The ability of monoclonal antibodies specifically to bind to antigens of microorganisms can provide many opportunities for diagnosis and treatment. Such specificity is a most important requirement for prompt and accurate analysis and/or diagnosis,

particularly in diagnosing the presence of diseases which require prompt treatment. In addition, the combination of a series of labeled monoclonal antibodies specific for different STDs in a kit system would allow not only a rapid, sensitive, and specific diagnosis, but a comprehensive diagnosis as well.

A wide variety of isotopic and nonisotopic immunoassays have been utilized in conjunction with monoclonal antibodies to test for the presence of antigenic substance. At the present time, agglutination, immuno-fluorescent, chemiluminescent or fluorescent immunoassay, immunoelectron microscopy, radiometric assay systems, radioimmunoassays, and enzyme-linked immunoassays are the most common techniques used with the monoclonal antibodies. Other techniques include bioluminescent, fluorescence polarization, and photon-counting immunoassays.

When utilizing the enzyme-linked immunoassay procedure (EIA), it is necessary to bind, or conjugate, the monoclonal antibody with an enzyme such as alkaline phosphatase capable of functioning in such assay.

The enzyme-linked monoclonal antibody can then be used in the known enzyme-linked immunosorbent assay procedure to determine the presence of an antigenic substance.

After detection of the specific antigen, the infecting organism can be determined and appropriate treatment can then be initiated to rapidly and efficiently eliminate the disease. In addition, the use of a comprehensive STD kit system would allow the detection of any "hidden" diseases, and treatment could then be initiated simultaneously with the primary infection.

While monoclonal antibody test kits against a particular infectious disease organism are known, a comprehensive STD kit comprising labeled monoclonal antibodies does not exist. A monoclonal antibody STD kit system would overcome many of the disadvantages of prior laboratory techniques in diagnosing the presence of STDs.

SUMMARY OF THE INVENTION

The present invention provides a monoclonal antibody test kit for use in accurately, rapidly, and completely diagnosing specimens for the presence of any agents capable of causing an STD.

Briefly stated, the present invention comprises a kit for the detection of all agents in a diagnostic specimen known to indicate a sexually transmitted disease comprising: (a) means containing a plurality of spaced apart reaction zones, and (b) a plurality of monoclonal antibody reagents for use with said means; there being at least one said monoclonal antibody reagent for each such agent capable of reacting in a detectable immunologic manner with said agent. In particular, the monoclonal antibody reagents combine with the infectious agents selected from Neisseria sp., Treponema sp., Chlamydia sp., Herpes sp., Trichomonas sp., Candida sp., and/or Gardnerella vaginalis, HTLV-III AIDS associated retrovirus, Mycoplasma sp., Giardia sp., Entamoeba sp., hepatitis sp., Salmonella sp., Shigella sp., Campylobacter sp., Mobiluncus sp., cytomegalovirus, Streptococcus sp., Staphylococcus aureus, Pneumocystis carynii, Bacteriodes fragilis, Legionella pneumophila, Hemophilus ducreyi, and Calymmatobacterium granulomatis. The labels for the reagents can be chosen from the group comprising radioactive

isotopes, enzymes, fluorescent compounds, chemiluminescent compounds, bioluminescent compounds, ferromagnetic atoms, or particles, or any other known label.

DETAILED DESCRIPTION

The production of monoclonal antibodies is a well-known procedure first described by Kohler and Milstein (Eur. J. Immunol. 6, 292 (1975)). In general, the monoclonal antibodies are prepared by fusing spleen cells from a mammal which has been immunized against a particular antigen or organism with an appropriate myeloma cell line. The fused cells yielding an antibody which gives a positive response to the presence of a particular organism or antigen are removed and cloned utilizing any of the standard methods. The monoclonal antibody selected, which is specific for a particular antigen or species, is then bound to an appropriate label.

Amounts of antibody sufficient for labeling and subsequent commercial production are produced by the known techniques, such as by batch or continuous tissue culture or culture in vivo in mammals, such as mice.

The monoclonal antibodies may be labeled with a multitude of different labels, such as enzymes, fluorescent compounds, luminescent compounds, radioactive compounds, ferromagnetic labels, and the like. The present invention will be described with reference to the use of an enzyme labeled monoclonal antibody. Some of the enzymes utilized as labels are alkaline phosphatase, glucose oxidase, galatosidase, peroxidase, or urease, and the like.

Such linkage with enzymes can be accomplished by any accepted method, such as the Staphylococcal Protein A method, the glutaraldehyde method, the benzoquinone method, or the periodate method.

Once the labeled monoclonal antibody is formed, testing is carried out employing one of a wide variety of conventional immunoassay methods. The particular method chosen will vary according to the monoclonal antibody and the label chosen. At the present time, enzyme immunoassays are preferred due to their low cost, reagent stability, safety, sensitivity, and ease of procedure. One example is enzyme-linked immunosorbent assay (EIA). EIA is a solid phase assay system which is similar in design to the radiometric assay, but utilizes an enzyme in place of a radioactive isotope as the immunoglobulin marker.

Fluorescent immunoassay is based on the labeling of antigen or antibody with fluorescent probes. A nonlabeled antigen and a specific antibody are combined with identical fluorescently labeled antigen. Both labeled and unlabeled antigen compete for antibody binding sites. The amount of labeled antigen bound to the antibody is dependent upon, and therefore a measurement of, the concentration of nonlabeled antigen. Examples of this particular type of fluorescent-immunoassay would include heterogeneous systems such as Enzyme-Linked Fluorescent Immunoassay, or homogeneous systems such as the Substrate Labeled Fluorescent Immunoassay. The most suitable fluorescent probe, and the one most widely used is fluorescein. While fluorescein can be subject to considerable interference from

scattering, sensitivity can be increased by the use of a fluorometer optimized for the probe utilized in the particular assay and in which the effect of scattering can be minimized.

In fluorescence polarization, a labeled sample is excited with polarized light and the degree of polarization of the emitted light is measured. As the antigen binds to the antibody its rotation slows down and the degree of polarization increases. Fluorescence polarization is simple, quick, and precise. However, at the present time its sensitivity is limited to the micromole per liter range and upper nanomole per liter range with respect to antigens in biological samples.

Luminescence is the emission of light by an atom or molecule as an electron is transferred to the ground state form a high energy state. In both chemiluminescent and bioluminescent reactions, the free energy required to produce an itermediate reaction or product in an electronically excited state. Subsequent decay back to the ground state is accompanied by emission of light. Bioluminescence is the name given to a special form of chemiluminescence found in biological systems, in which catalytic protein or enzyme, such a luciferase, increases the efficiency of the luminescent reaction. Among the most common chemiluminescent substances is luminol.

Once the labeled monoclonal antibodies are prepared, they can be assembled into a diagnostic kit for use in detecting the presence of an antigen or organism. The present invention provides for a plurality of monoclonal antibody reagents each capable of reacting in a desirable manner with only one of the following infectious agents: Neisseria, Treponema, Chlamydia, Herpes, Trichomonas, Candida,

Gardnerella vaginalis, HTLV-III or AIDS associated retrovirus, Mycoplasma, Giardia, Entamoeba, hepatitis, Salmonella, Shigella, Campylobacter, Mobiluncus, cytomegalovirus, Group B Streptococcus, Staphylococcus aureus, Pneumocystis carynii, Bacteriodes fragilis, Legionella pneumophila, Hemophilus ducreyi, and Calymmatobacterium granulomatis. In particular, the kit would comprise the reagents capable of indicating the presence of a STD. For example, the presence of Neisseria gonorrhoeae would indicate gonorrhoea; Treponema pallidum would indicate syphilis; or HTLV-III virus or antibody would indicate a high risk of contracting AIDS.

As used herein the phrase "all agents in a diagnostic specimen known to indicate a sexually transmitted disease" means those agents presently accepted by most authorities as causing a sexually transmitted disease (STD) as well as those agents which are hereafter accepted as causing an STD. Moreover, it will be understood that the kits may vary in composition based on the part of the United Statees or world since it is recognized that the "universe" of agents causing STD's in any region may vary widely from that of other regions. Thus, for example, in some regions Legionella may not be present as a cause of STD and hence need not be included as part of the kit.

The means containing a plurality of spaced apart reaction zones can be any presently available immunologic test device having a sufficient number of separate reaction zones. Preferred means are any one of the commercially available polymeric microtiter trays having as many as 96 wells. Such devices are capable of absorbing labeled monoclonal antibodies on their polymeric structure, as in the wells. Different wells would contain different monoclonal antibodies. For

instance, one kit would contain labeled monoclonal antibodies to all of the species of the following organisms known to cause STD: Neisseria gonorrhoeae, Treponema pallidum, Herpes hominus, Gardnerella vaginalis, Candida albicans, Chlamydia trachomatis, Trichomonas vaginalis, human T-Lymphotrophic virus - III, Mycoplasma hominis, Guardia lamblia, Entamoeba histolytica, hepatitis B, Salmonella, Shigella, Campylobacter, Mobiluncus, cytomegalovirus, Group B Streptococcus, Staphylococcus aureus, Pneumocystis carynii, Bacteriodes fragilis, Legionella pneumophila, Hemophilus ducreyi, and Calymatobacterium granulomatis or any combination of the above. The presence of these agents or their antigens can then be determined by exposing the venereal disease plate to suitably prepared specimens of urine, pus, stool, or the like. A positive test is indicated by a characteristic color appearing in at least one of the microtiter wells. The presence of two positive tests would indicate two different venereal diseases. For instance, if there are two wells, each of which contain an alkaline-phosphatase labeled monoclonal antibody, one of which will combine in an immunological manner with Neisseria gonorrhoeae and the other with Treponema pallidum, both diseases are indicated by a characteristic yellow color appearing in each particular well upon exposure to a specimen.

Other embodiments of the present invention include immobilizing the different labeled monoclonal antibodies on a particular section of a planar sheet. The present invention contemplates a different labeled monoclonal antibody on each segment of the sheet capable of contacting different venereal diseases. The sheet can be made of

paper, plastic, or other suitable material; being necessary only that it be a surface to which the labeled monoclonal antibody can be made to adhere.

Alternatively, a plurality of filter matrices can have immobilized on or within their surfaces the different monoclonal antibodies. The other components are added to the filter matrix and after reaction, unbound unreacted materials are removed by washing and the signal read visually or by instrumentation means.

The present invention contemplates any number of different immunoassay techniques and/or substrates, and these examples are not intended to limit the application to any particular procedure.

In the past, there have been difficulties in developing rapid kits because of undesirable cross-reactions of specimens with antiserum. The use of monoclonal antibodies can eliminate these problems and provide highly specific and rapid tests for diagnosis. A rapid and precise kit could replace or augment existing tests and permit appropriate therapy to be initiated early. In addition, utilization of a comprehensive STD kit, as in Applicant's invention, provides for the rapid detection of most possible sexually transmitted organisms.

The STD kit can be used on an out-patient basis, or as part of an automated laboratory procedure. At present, the lack of a rapid test giving "same day" results may delay the initiation of treatment and complicate the clinical management of a patient with more severe symptoms. A comprehensive test kit that provides complete results within one to two hours and discloses the presence of any infectious agent capable of causing an STD will expedite treatment of patients.

Further embodiments of the present invention would include a kit comprising a plurality of monoclonal antibody reagents known to indicate a STD, as well as control samples of particular STD organisms. The control samples could be used to determine the shelf-life of the kit.

The invention will be further illustrated in connection with the following examples which are set forth for purposes of illustration only and not by way of limitation.

EXAMPLES

EXAMPLE I

A. Procedure for preparing a microtiter tray

Individual wells of a 96-well microtiter tray (Flow Laboratories, Inc., McLean, Virginia) are each coated with 1-10 ug/ml (in a carbonate buffer pH 8.75) of monoclonal antibody directed against Neisseria gonorrhoeae, Treponema pallidum, Chlamydia trachomatis, Herpes hominus, Trichomonas, vaginatis, Candida albicans, Gardnerella vaginalis, AIDS or AIDS-associated retrovirus (HTLV-III) or its antibody, Mycoplasma hominis, Giardia lamblia, Entamoeba histolytica hepatitis B, Salmonella, Shigella, Campylobacter, Mobiluncus, cytomegalovirus, Group B Streptococcus, Staphylococcus aureus, Pneumocystis carynii, Bacteriodes fragilis, Legionella pneumophila, Hemophilus ducreyi, and Calymmatobacterium granulomatis such that each well is coated with a different monoclonal antibody specific for each one of the infective agents listed. The tray is incubated from 1 hour at 37°C to 24 hours at

4°C. The incubated material is removed and the tray washed with 0.05M Tris buffered saline pH 7.4, 0.3M NaCl & 0.1% BSA. A blocking agent (bovine serum albumin in 0.05% Tween) is added to the tray wells to block uncoated plastic surface areas.

B. Procedure for assay - sandwich method

To each coated well of the microtiter tray of step A is added an aliquot of specimen, such as a vaginal smear or swab extract, and allowed to incubate for 1 hour at 37C. The tray is washed with Tris buffered saline solution with a BSA solution to remove unbound specimen. Then, to each well is added 100ul of a mixture composed of monoclonal antibodies each directed against one of the antigens listed in step A, each monoclonal antibody being conjugated with alkaline phosphatase as a label. The tray is washed with buffer solution to remove unbound labelled monoclonal antibody. To each well is added 100ul of indoxyl phosphate substrate. A visible blue color will appear in each well where the particular antigen is present and has reacted with the monoclonal antibodies, affording a visual indication of which venereal or sexually transmitted diseases are present in the patient.

While the invention has been described in connection with certain preferred embodiments, it is not intended to limit the scope of the invention to the particular form set forth, but, on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A kit for the detection of all agents in a diagnostic specimen known to indicate a sexually transmitted disease comprising:

(a) means containing a plurality of spaced apart reaction zones, and

(b) a plurality of monoclonal antibody reagents for use with said means; there being at least one said monoclonal antibody reagent for each such agent capable of reacting in a detectable immunologic manner with said agent.

2. The kit of Claim 1 wherein said kit includes at least one monoclonal antibody reagent for each of the infectious agents selected from Neisseria sp., Treponema sp., Chlamydia sp., Herpes sp., Trichomonas sp., Candida sp., Gardnerella vaginalis, AIDS or AIDS associated retrovirus sp., mycoplasma sp., Giardia sp., Entamoeba sp., hepatitis sp., Salmonella sp., Shigella sp., Campylobacter sp., Mobiluncus sp., cytomegalovirus sp., Group B Streptococcus sp., Staphylococcus aureus, Pneumocystis carynii, Bacteriodes fragilis, Legionella pneumophila, Hemophilus ducryei, and Calymmatobacterium granulomatis.

3. The kit of Claim 1 wherein said plurality of monoclonal antibody reagents are immobilized within said plurality of spaced apart reaction zones such that a different monoclonal antibody is present in each of said zones.

4. The kit of Claims 1, 2 or 3 wherein said at least one monoclonal antibody reagent is labeled.

5. The kit of Claim 4 wherein said at least one monoclonal antibody reagent is labeled with a fluorescent compound.

6. The kit of Claim 5 wherein said fluorescent compound is fluorescein.

7. The kit of Claim 4 wherein said at least one monoclonal antibody reagent is labeled with an enzyme.

8. The kit of Claim 7 wherein said enzyme is selected from alkaline phospatase, glucose oxidase, galactosidase, or peroxidase.

9. The kit of Claim 4 wherein said at least one monoclonal antibody reagent is labeled with a chemiluminescent compound.

10. The kit of Claim 9 wherein said chemiluminescent compound is luminol or a luminol derivative.

11. The kit of Claim 4 wherein said at least one monoclonal antibody reagent is labeled with a bioluminescent compound.

12. The kit of Claim 10 wherein said bioluminescent compound is luciferase or a luciferase derivative.

13. The kit of Claim 4 wherein said at least one monoclonal antibody reagent is labeled with a member of the group consisting of a radioactive isotope, ferromagnetic atom, or particle.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/00892

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4) C12Q 1/26,34,42,70;G01N 33/53,544,545
USCl. 435/5,7,18,21,25,28,810;436/510,528,531,548,800,804,806,809,811

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US Cl	435/5,7,18,21,25,28,810 436/510,528,531,548,800,804,806,809,811

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

STN INTERNATIONAL - FILECA, FILE BIOSIS: "SEXUALLY (W) "TRANSMITTED (W) DISEASE?", "MULTIPLE", "SIMULTANEOUS", "MICROTIT?", "MONOCLONAL".

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	Journal of Clinical Microbiology, Volume 17, no. 6, issued 1983 June (Washington, D.C.), J. BLOOMBERG et al, "Viral Antibody Screening System That Uses a Standardized Single Dilution Immunoglobulin G Enzyme Immunoassay with Multiple Antigens", pages 1081-1091 (see abstract).	1-13
Y	Journal of Clinical Microbiology, Volume 20, no. 6, issued 1984 December (Washington, D.C.) C.E. FARSHY et al. "Double-Conjugate Enzyme-Linked Immunosorbent Assay for Immunoglobulins G and M Against Treponema palladium", pages 1109-1113 (see abstract).	1
Y	EP, A, 0173295 (New York Blood Center) 05 March 1986 (05.03.86) See pages 2 and 3.	1-13

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
26 JUNE 1987	13 JUL 1987
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Christine M. Nucker

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
Y	US, A, 4,378,344 (R. J. ZAHRADNIK et al) 29 March 1983 (29.03.83), see column 2, lines 22-36; column 10, lines 38-47.	1-13
Y,P	US, A, 4,514,508 (S. HIRSHFELD) 30 April 1985 (30.04.85) see abstract and column 5, lines 3-16.	1-13
Y	US, A, 4,514,509 (R.B. KOHLER et al) 30 April 1985 (30.04.85) see abstract.	1
Y	US, A, 4,520,113 (R.C. GALLO et al) 28 May 1985 (28.05.85) see abstract.	1
Y,E	US, A, 4,663,227 (C.G. WANG) 05 May 1987 (05.05.87) see column 8, lines 18-36.	1
A	US, A, 4,486,530 (G.S. DAVID et al) 04 December 1984 (04.12.84).	