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(54) Title: DEVICE AND METHOD FOR DETERMINING MICROBIAL RESISTANCE TO ANTIMICROBIC AGENTS

(57) Abstract

A device and method for detecting and identifying an antimicrobic agent modifying entity selected from enzymes or blocking or binding proteins, comprising a means for retaining at least two antibodies selected from monoclonal antibodies, polyclonal antibodies, or mixtures thereof in spaced relationship to each other, contacting a specimen suspected of containing an antimicrobic entity with the at least two antibodies, and determining when a reaction has taken place between the antibodies and the entity by means of any immunoassay technique suitable for use with at least two antibodies.

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DEVICE AND METHOD FOR DETERMINING MICROBIAL RESISTANCE TO ANTIMICROBIC AGENTS

BACKGROUND OF THE INVENTION

An important procedure in combating a microbial infection is the determination of the causative organism or organisms and which antimicrobial agent is most effective in destroying or inhibiting the organism or organisms. At present no rapid accurate test exists for either of these determinations. This problem is further made difficult by the fact that any given infective organism may be resistant to the particular antibiotic of choice and consequently resistance to antibiotics need also to be determined.

Beta-lactam antibiotics bind to primary receptors, known as membrane associated penicillin-binding proteins (PBP's), which play a central role in the cell cycle-related, morphogenetic synthesis of cell wall peptidoglycan. Inactivation of PBP's by antibiotics has an immediate impact on the bacterial cell. Mutation of the bacterial genes which express a particular PBP can result in the resistance to inactivation by beta-lactam antibiotics. Among the best known illustrations of clinically important resistance to beta-lactam antibiotics dependent on PBP alternations are penicillin resistance in gonococcus and methicillin resistance in S. Aureus.

The variety and number of beta-lactamases have been a perplexing problem for physicians. So to has the recent finding that the genes controlling beta-lactamase production can be transferred by plasmids from one bacterial species to another.

Among other mechanisms of antimicrobic agent and antibiotic resistance, microorganisms such as bacteria may express enzymes which structurally alter or inactivate certain antibiotics or other antimicrobic agents which are used to treat infections caused by such

bacteria. Common among these antibiotic modifying enzymes are the beta-lactamases which can hydrolyze the lactam ring of beta-lactam antibiotics, such as the penicillins and cephalosporins and render them ineffective.

In an effort to counter this mechanism of resistance, new beta-lactam drugs have been developed with structures intended to be resistant to the action of the commonly produced beta-lactamases. Some bacteria have responded with new forms of beta-lactamase which are capable of hydrolysing even the most defensively structured beta-lactam antibiotics. These enzymes are largely responsible for bacterial resistance to the penicillin and cephalosporin family of antibiotics.

Another family of antibiotics, the aminoglycoside-aminocyclitols, such as Neamine, Neomycin, Gentamicin, Tobramycin, Streptomycin and Sorbistin are also subject to inactivation by the bacteria-produced plasmid encoded enzymes which may phosphorylate, adenylate, or acetylate the drug's structure, and eliminate or reduce the antimicrobic activity of this important class of antibiotics.

In like manner, other antimicrobic agents such as polypeptides (e.g., Bacitracin, which inhibits peptidogycan synthesis by inhibiting the dephosphorylation of used pyrophosphate to lipid phosphate, a step essential to the regeneration of the lipid carrier) can also be rendered inactive.

Other classes of antimicrobics which may be inactivated include macrolides (e.g., Erythromycin, Clindamycin and Lincomycin), quinolones (e.g., Ciprofloxacin, Norfloxacin and nalidixic acid), thienamycins (e.g., Imipenem) and the like.

In more passive action of resistance to antimicrobic agents, bacteria may express proteins which appear to block or bind to an antibiotic agent and sterically hinder their mode of action.

Present testing methods include culturing the bacteria in the presence of the antibiotic for a period of time, normally 4-18 hours, and observing the bacteria's ability to reproduce and multiply in the presence of the antibiotic. In these culture tests, the exact mode of antibiotic resistance is seldom known.

Other methods have employed a chromogenic beta-lactam antibiotic, which produces a color change when hydrolyzed.

U.S. Patent No. 4,383,032, issued 10 May 1983 to Stahl et al., discloses a reagent and process for the determination of beta-lactamase using 7-cyanoacetylaminocephalosporanic acid and a mixture of ammonium ions, phosphate ions and oxygen-splitting agents. A red color forms where beta-lactamases are present.

U.S. Patent No. 4,381,343, issued 26 April 1983 to Citri, describes a technique for the determination of antibacterial agents wherein a sample containing a beta-lactum antibiotic is applied to a site on a nutrient medium that has been seeded with beta-lactamase generating bacteria or spores; incubating the medium to promote the generation of beta-lactamase; and, assaying the beta-lactamase produced. Since the induction of beta-lactamase production is specific to beta-lactam antibiotics, the test provides an indication of the presence or amount of beta-lactam antibiotics in the sample. The invention is limited in the sensitivity obtainable, which appears to be substantially less than for typical fluorescent enzyme immunoassay techniques.

U.S. Patent No. 4,234,683, issued 18 November 1980 to McMillan, describes another method using starch and iodine to detect the production of penicilloic acid from enzymatic hydrolysis of penicillin. In the assay, the normally blue/black starch iodine complex is blocked by the penicilloic acid and the solution clears, reflecting the presence of one of the beta-lactamases. This assay, however, is not very sensitive and not all beta-lactam antibiotics produce the necessary penicilloic acid when hydrolyzed.

None of the currently known beta-lactamase test can sensitively detect all beta-lactamases produced by bacteria; no practical tests are available for the detection of the aminoglycoside-modifying enzymes; and no practical tests are available to detect other proteins which block, bind, or otherwise inhibit the antimicrobial activity of antimicrobic agents.

Also, and most importantly, no single test exists which can utilize a single specimen to detect microbial resistance regardless of the nature of the antimicrobic agent or of the nature of any inactivating enzymes or other proteins.

SUMMARY OF THE INVENTION

The present invention provides a test device and method employing a series of monoclonal or polyclonal antibodies in an immunoassay to detect and identify antimicrobic agent modifying enzymes and antimicrobic agent blocking or binding proteins as are produced by pathogenic microorganisms.

Briefly, the present invention comprises a method for detecting and identifying antimicrobic agent modifying enzymes or antimicrobic agent blocking or binding proteins in a specimen comprising contacting a specimen with at least two antibodies selected from monoclonal antibodies, polyclonal antibodies, or mixtures thereof arranged in a spaced relationship to each other and determining when a reaction has taken place between any of said at least two antibodies and an antimicrobic agent modifying agent or antimicrobic agent blocking or binding protein in said specimen by means of any immunoassay suitable for use with said antibodies.

The test device that is also the subject of the present invention is described more fully below.

DETAILED DESCRIPTION

A panel of immunoassays to detect the presence of antibiotic modifying enzymes and other antibiotic reactive proteins, as are expressed by bacteria and perhaps other microorganisms, can accurately and quickly predict resistance and thereby infer susceptibility of microorganisms to antimicrobic therapy. The

time necessary to perform such assays is a matter of minutes, compared to many hours as required of the currently employed antimicrobic susceptibility tests using bacterial culture methods.

In practice, the microorganism being tested are exposed to antibodies to the antimicrobic modifying enzymes or proteins, and the presence of such enzymes or proteins is detected by the resulting immunochemical reaction. The immunochemical reaction employs conventional methods, including, but not limited to, fluorescence, luminescence, radioisotope emission and spectral absorbence, and enzyme amplified variations of such methods.

In some instances, it may be necessary to stimulate expression or production of the antimicrobic modifying enzyme or protein by the microorganism being tested. In such cases, the microorgansm will be exposed to sublethal concentrations of antimicrobics, prior to or during testing for the antimicrobic modifying enzyme or protein, to induce the microorganism to produce such enzyme or protein if it possesses the genetic coding or capability to do so.

The immunoassay may employ any conventional heterogenous technique wherein an antibody to the antibiotic modifying enzyme or protein is bound to a solid support, such as plastic, latex or cellulose materials, or the like, and the test organism or a specimen containing the metabolites is exposed to the solid-bound antibody.

If the microbial material contains the enzyme or protein to which the antibody is active, such enzyme or protein will be bound by the antibody. Extraneous, unreacted microbial material and metabolite is then washed off or otherwise removed from the solid support and the antibody/enzyme and antibody/protein complexes bound to the solid support. A second antibody to the same enzyme or protein, which is labelled or tagged with a compound or molecule or isotope and is

detectable by visual or instrumented observations, is added and exposed to the solid bound antibody/enzyme or antibody/protein complex and will, in turn, react with and bind to the enzyme or protein. Unbound second antibody is then washed off or otherwise removed from the resulting solid bound antibody/enzyme labelled antibody or antibody/protein/labelled antibody complexes. The labelling compound or molecule or isotope is then detected by the analytical method for which it was intended, and the detectable presence of the labelling substance is indicative of the presence of the antibiotic modifying enzyme or protein. The amount of labelling substance bound to the solid support is quantitatively proportional to the amount of enzyme or protein in the test organism or metabolite solution.

An example of a heterogeneous assay employs a set or series of devices whereby a specimen is contacted with insolubilized antibody and a labelled second antibody, both of which are directed against the modifying entity. The reaction mixture is performed on or within or is subsequently added to a filter means and washed to effect a separation of bound from unbound material. In the case of an enzyme label an appropriate substrate is added to develop a colored product, if a complex has formed, indicating the presence of antibmicrobic agent modifying enzyme or protein. A panel of discrete reactions can be achieved using either a number of single reaction filter devices or one device adapted for multiple reactions. Several such assay systems are commercially available.

A kit can be used incorporating reagents for a test, such as a heterogeneous assay, using insolubilized antibody, labelled second antibody conjugate, wash solution, and a multicavity filter device. Another kit assembly that can be used comprises a conventional

microtiter tray with a first antibody captured thereon with a different first antibody in each well, thereby forming a panel of different tests, a labelled second antibody and wash solution.

Selection of appropriate antibodies is also relevant to assay performance. In a conventional sandwich-type assay employing a capture antibody and a labelled antibody, one possible arrangement using a array of discrete reaction zones in a spatial relationship, such as a microtiter tray, would have as the capture antibody a polyclonal antibody broadly directed against antibodtic modifying enzymes or proteins and a different second labelled monoclonal antibody for different modifying enzymes or proteins comprising a panel of tests. This second labelled antibody could be added as a mixture of monoclonal antibodies, thus enabling a user to add the labelled antibody as a single solution rather than individually add numerous different labelled antibodies. Alternatively, each reaction zone could have a different monoclonal antibody contained on or within it, and after adding specimen, would have a labelled polyclonal antibody solution added. The invention is flexible so as to permit these, as well as other procedural steps to be chosen as warranted for the circumstances.

The immunoassay may also employ any conventional homogenous technique in which the test organism or metabolite specimen is mixed with labelled antibody to the antibiotic modifying enzyme or protein, and the resulting immunochemical reaction is detected by a change in the chemical or photometric activity of the labelling compound, which change is induced by the presence or absence of antibiotic modifying enzyme or protein. The amount of change in the chemical or

photometric activity of the labelling compound is quantitatively proportional to the amount of enzyme or protein in the test organism or metabolite solution.

The particular immunoassay procedure used depends mainly on that which is most effective with any specific antibody or antibodies in giving rapid and accurate results. The most suitable immunoassay can be easily determined by routine testing using the antibody or antibodies with various known immunoassay procedures against standard antigens (enzymes and blocking or binding proteins) and choosing that which is most effective.

The test organism or metabolite solution is either contained in the specimen (urine, blood, wound exudate, cerebral spinal fluid, serum or plasma, saliva, feces, sputum, mucus, pus, or other body fluid or tissue) which is collected from or near the site of infection, or which is subsequently isolated or extracted from such material by additional processing.

The antimicrobic modifying enzymes or protein may also be detected directly in the body fluid or tissue of the infected patient, without collection or isolation of the infection organism. In such test the patient's urine, blood, wound exudate, spinal fluid, or other body fluid or tissue is tested by the immunoassay for the presence of antimicrobic agent modifying enzymes or proteins, without regard to the presence of the infecting organism in the test material. The absence or presence of such antimicrobic agent modifying enzymes or proteins is the information required by a physician to determine which antibiotic to utilize in treating the patient.

The labelling material can be any material capable of emitting a detectable signal, such as, but not limited to a radioactive isotope, an enzyme, fluorescent, bioluminescent, chemiluminescent material or ferromagnetic atom or particle.

The invention will be further described in connection with the following examples which are set forth for purposes of illustration only.

EXAMPLES

EXAMPLE I

A. Procedure for Preparing a Monoclonal Antibody Against Penicillinase:

This antibody is prepared according to the general procedure disclosed by Milstein & Kohler in NATURE 256:495-497, 1975. The monoclonal antibodies of the present invention are prepared by fusing spleen cells, from a mammal which has been immunized against penicillinase, with an appropriate myeloma cell line such as NS-0. The resultant product is then cultured in a standard HAT (hypoxanthine, aminopterin, and thymidine) medium. Screening tests for the specific monoclonal antibodies are employed utilizing immunoassay techniques which will be described below.

The immune spleen cells may be derived from any mammal, such as primates, humans, rodents (i.e., mice, rats, and rabbits), bovine, ovine, canine, or the like, but the present invention will be described in connection with mice. The mouse is first immunized by injection of the antigen chosen generally for a period of eleven weeks. When the mouse shows sufficient antibody production against the antigen, as determined by conventional assay, it is given a

booster injection of the antigen, and then killed so that the spleen may be removed. The fusion can then be carried out utilizing immune spleen cells and an appropriate myeloma cell line.

The fused cells yielding an antibody which give a positive response to the presence of the antigen are removed and cloned utilizing any of the standard methods. The monoclonal antibodies from the clones are then tested against standard antigens to determine their specificity for the particular antigen. The monoclonal antibody selected, which is specific for the antigen or species, is then bound to an appropriate label.

Amounts of antibody sufficient for labelling 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 labelled with a multitude of different labels, such enzymes, fluorescent compounds, luminescent compounds, radioactive compounds, ferromagnetic labels, and the like. Some of the enzymes utilized as labels are alkaline phosphatase, glucose oxidase, galactosidase, peroxidase, urease, and the like.

Such linkage with enzymes can be accomplished by any one of the conventional and known methods, such as the Staphylococcal Protein A method, the glutaraldehyde method, the benzoquinone method, or the periodate method.

Other monoclonal antibodies are prepared similarly by substituting for penicillinase an enzyme or protein of interest:

B. Procedure for preparation of reaction container:

A 96-well microtiter tray (Flow Laboratories, Inc., McLean, Virginia) is coated with a 1-10 ug/ml in a 0.5M carbonate buffer (pH 9.7) of a monoclonal antibody directed against an enzyme or

protein capable of modifying or inactivating penicillin, cephalosporin, amikacin, Gentamycin, Neomycin, Erythromycin, Clindamycin, Lincomycin, Ciprofloxacin, Norfloxacin, nalidixic acid and Imipenem, such that each well contains a different monoclonal antibody for each modifying enzyme or protein. The tray is incubated for 1 hour at 37C or 24 hours at 4C. The incubated material is removed and the tray washed with 0.05M Tris buffered saline, pH 7.4, 0.3M NaCl and 0.1% BSA. A blocking agent (bovine serum albumin in 0.05% Tween) is added to the tray wells to block any uncoated plastic surface areas.

C. 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 blood or urine sample, and allowed to incubate for 1 hour at 37C. The tray is washed with Tris buffered saline with 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 enzyme or proteins listed in step A, each monoclonal antibody being conjugated with alkaline phosphatase as a label. The tray is washed with Tris buffered saline with BSA 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 antimicrobic agent modifying enzyme or protein is present.

EXAMPLE 2

A. Procedure for preparing monoclonal antibodies to antimicrobic agent modifying enzymes or proteins:

The procedure according to Example 1, Step A is followed wherein substituted for penicillinase is an enzyme or protein listed in Example 1, Step B.

B. Procedure for assay in a filter device:

To a multicavity or multichamber filter device such as that described in co-pending Application No. 740,100 is added 1 drop (50ul) of each monoclonal antibody prepared in Step A above, which have been coated on latex particles (0.5% in glycine buffer) such that each distinct reaction zone receives one distinct type of monoclonal antibody. 1.0 ml of specimen is added to each reaction zone of the filter device. Then 1 drop (50ul) of a second monoclonal antibody directed against the particular modifying enzyme or protein, whereby the monoclonal antibody is conjugated with alkaline phosphatase (in Tris buffer, containing bovine serum albumin and Mg) is added to each corresponding reaction zone of the filter device. The reaction zones are incubated from 1 minute to 1 hour, and then washed with 2ml of 0.05 M Tris/0.5% gelatin buffer solution. Two drops of 2 mg/ml indoxyl phosphate in glycine buffer are added, and incubation is allowed for several minutes. If the specimen is positive for the presence of the particular antimicrobic agent modifying enzyme or protein, a blue color will appear. The color development can be stopped if desired with 1 ml 1N HCl.

EXAMPLE 3

The procedure according to Example 2 is followed, wherein the respective and corresponding first and labelled monoclonal antibodies are combined together with the specimen and allowed to react, and then added to the filter device to each respective reaction zone.

While the invention has been described in connection with a preferred embodiment, 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 method for detecting or identifying the presence in a specimen of an antimicrobic agent modifying entity selected from enzymes or blocking or binding proteins comprising contacting said specimen with at least two antibodies selected from monoclonal antibodies, polyclonal antibodies, or mixtures thereof arranged in a spaced relationship to each other and determining when a reaction has taken place between any of said at least two antibodies and any said entity in said specimen by means of any immunoassay generating a detectable signal and suitable for use with said at least two antibodies.

2. A heterogeneous method for detecting or identifying the presence of a specimen of an antimicrobic agent modifying entity selected from enzymes or blocking or binding proteins, comprising:

(a) contacting with said specimen at least two insolubilized antibodies directed against said enzymes or proteins said antibodies arranged in a spaced relationship to each other and at least one labelled antibody directed against said enzymes or proteins in a manner favoring the formation of a complex;

(b) separating bound from unbound material; and

(c) determining the activity of said label as being correlative with the presence or amount of said antimicrobial agent modifying entity in said specimen.

3. A competitive method for detecting or identifying the presence in a specimen of an antimicrobic agent modifying entity selected from enzymes or blocking or binding proteins, comprising:

(a) contacting with said specimen a known amount of an antimicrobic agent modifying entity conjugated to a signal generating label and at least two insolubilized antibodies containing a number of binding sites so that said specimen and said labelled antimicrobic agent modifying entity compete for binding sites on said at least two insolubilized antibodies;

(b) separating bound from unbound material; and

(c) measuring the activity of any of said label present as being inversely related to the presence or amount of said antimicrobic agent modifying entity.

4. A homogeneous method for detecting or identifying the presence in a specimen of an antimicrobic agent modifying entity selected from enzymes or blocking or binding proteins suspected of being in a specimen, comprising:

(a) contacting said specimen with an effective amount of at least two antibodies each conjugated with a different label; and

(b) measuring the change in signal emitted by said label when bound to any of said modifying entity as an indication of the presence of said modifying entity.

5. The method of claims 1, 2, 3 or 4 wherein said at least two antibodies are polyclonal antibodies.

6. The method of claims 1, 2, 3 or 4 wherein said at least two antibodies are monoclonal antibodies.

7. The method of claims 1, 2, 3 or 4 wherein said at least two antibodies are selected from a mixture of monoclonal and polyclonal antibodies.

8. The method of claims 1, 2, 3 or 4 wherein said specimen is a biological fluid selected from whole blood, serum or plasma, saliva, feces, sputum, mucus, cerebral spinal fluid, urine, cell or tissue extracts, pus, or wound exudate.

9. The method of claims 1, 2, 3 or 4 wherein said label is selected from a radioactive isotope, an enzyme, fluorescent, bioluminescent, or chemiluminescent material or ferromagnetic atom or particle.

10. The method of claim 1, 2, 3 or 4 wherein said labels are composed of a fluorescent material such that each type of label emits energy of a unique and detectable characteristic wavelength.

11. The method of claim 1, 2, 3 or 4 wherein at least two antibodies are a mixture of different and distinct labelled monoclonal antibodies contacted with said specimen simultaneously.

12. The method of claim 1, 2, 3 or 4 where said at least two antibodies are ~~monoclonal~~ antibodies each of which is directed against a different and distinct antimicrobic agent modifying entity.

13. An immunoassay test device for detecting or identifying the presence in a specimen of an antimicrobic agent modifying entity selected from enzymes or blocking or binding proteins comprising means for retaining at least two antibodies selected from monoclonal antibodies, polyclonal antibodies, or mixtures thereof in spaced relationship to each other and means associated with said at least two antibodies capable of giving a detectable immunological response when a reaction has taken place between any of said at least two antibodies and any said entity; said at least two antibodies being selected from antibodies to an antimicrobic agent modifying enzyme or antimicrobic agent blocking or binding protein.

14. A diagnostic test kit for detecting or identifying the presence in a specimen of an antimicrobic agent modifying entity selected from enzymes or blocking or binding proteins comprising at least two antibodies selected from monoclonal antibodies, polyclonal antibodies or mixtures thereof, means for retaining said at least two antibodies in spaced relationship to each other and means associated with said at least two antibodies capable of giving a detectable immunological response when a reaction has taken place between any of said at least two antibodies and any of said entity.

15. The test kit of claim 14 wherein said at least two antibodies are directed against said antimicrobic agent modifying entity.

16. The test of claim 14 wherein said entity is a beta-lactamase.

17. The test kit of claim 14 wherein said entity is an aminoglycoside-aminocylitol modifying enzyme.

18. The test kit of claim 14 wherein said antimicrobic agent is selected from a beta-lactam, aminoglycoside-aminocyclitol, macrolide, quinolone, or thienamycin.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/00570

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): G01N 33/53, 33/554, 33/577

U.S. CL: 435/7;436/523,526,533,534,536,548,804,808

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/7; 436/523,526,533,534,536,548,804,808

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

CHEMICAL ABSTRACTS 1977- JUNE 1986 UNDER "PENICILLINASE"

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	US,A, 4,177,253 (DAVIES) 04 December 1979, see claims 1 and 22	9
A	US,A, 4,234,683 (MCMILLAN) 18 November 1980, see Abstract	
Y	US,A, 4,361,647 (REMINGTON) 30 November 1982, see claim 1	7
Y	US,A, 4,376,110 (DAVID) 08 March 1983, see Abstract and claim 19	1-18
A	US,A, 4,381,343 (CITRI) 26 April 1983, see Abstract	

* 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]
19 May 1987	01 JUN 1987
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	Sidney Marantz

Form PCT/ISA/210 (second sheet) (May 1986)

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]
A	US,A, 4,383,032 (STAHL) 10 May 1983, see Abstract	
A	US,A, 4,443,549 (SADOWSKI) 17 April 1984, see Abstract	
Y	US,A, 4,474,892 (MURAD) 02 October 1984, see Abstract	1-18
Y	US,A, 4,514,505 (CANFIELD) 30 April 1985, see Abstract	7
Y	US,A, 4,596,769 (SHOCKMAN) 24 June 1986, see Abstract	1-18
A	J. Immunol. Methods, Vol. 83, No. 2, issued 1985 (Amsterdam, The Netherlands), R.R. Premier et al., "An Evaluation... Detection of Beta-Lactamase in Enzyme Immunoassay", pages 371-377, see Summary	