Patent Right	Date	November 6, 2018	Court	Intellectual Property High Court, Third Division
	Case number	2017 (Gyo-Ke) 10117		

- A case in which the court determines that "an invention described in a publication" of Article 29, paragraph (1), item (iii) of the Patent Act should constitute a basis for the determination of whether a person skilled in the art could have easily conceived of the present invention (the patent invention) on the basis of the state of the art as of the filing, and thus should be a specific technical idea that could be extracted from the description of the publication, and further it is required that a person skilled in the art could make a product on the basis of the description of the publication and the common general knowledge as of the filing of the Patent to find that the publication describes an invention of the product.
- A case in which the court determines that it cannot be recognized from the description of the cited reference and the common general knowledge as of the filing that the cited reference describes or substantially describes the cited invention, but the revocation decision of the patent is illegal in that it has made errors in finding of a fact of the cited invention and it has overlooked the different features.

Case type: Rescission of Patent Revocation Decision

Result: Granted

References: Article 29, paragraph (2) of the Patent Act

Numbers of related rights, etc.: Patent No. 5845033, Opposition No. 2016-700611

Summary of the Judgment

1 The case is a case in which an opposition to a granted patent of Patent No. 5845033, titled "IMMUNOCHROMATOGRAPHY TEST DEVICE AND KIT FOR MYCOPLASMA PNEUMONIAE DETECTION" was filed and the Japan Patent Office made a decision to the effect that the patent should be revoked, and thus the patentee has filed a suit against trial decision made by the JPO.

The reason for the JPO decision revoking a patent is violation of Article 29, paragraph (2) of the Patent Act (Lack of Inventive step). Plaintiff alleges [i] Errors in the finding of cited invention as well as the identical features and the different features, [ii] Errors in the determination of the different features, and [iii] Errors in the finding of significant function and effect.

2 The court decision has rescinded the JPO decision in summary as follows.

(1) The case in which the court determines that "an invention described in a publication" of Article 29, paragraph (1), item (iii) of the Patent Act should constitute a basis for the determination of whether a person skilled in the art could have easily conceived of the present invention (the patent invention) on the basis of the state of the art as of the filing, and thus should be a specific technical idea that could be extracted from the description of the publication. Further, the patent invention is an invention

of a product, and thus a comparison is made with an invention of a product described in a publication in considering the inventive step. Here, it is necessary for a person skilled in the art to be able to make a product on the basis of the description of the publication and the common general knowledge as of the filing of the Patent (hereinafter referred to as "as of the filing") in order to find that an invention of a product is described in the publication.

(2) A consideration is given to this case from such viewpoint that it cannot be said that the device of the cited invention 1 can be produced by a person skilled in the art even in view of the description of Cited reference 1 and the common general knowledge as of the filing. Specifically,

A The revocation decision finds the cited invention 1 as an invention directed to a lateral flow device that implements the detection of Mycoplasma pneumoniae in a patient's sample by use of a monoclonal antibody against P1 protein. The lateral flow device is a detection device based on immunochromatography. In the antigen detection by immunochromatography, it is essential to form a sandwich complex between an antibody and an antigen. Further, in a case of monoclonal antibody, it is inconvenient if two antibodies sandwiching an antigen are the same. Thus it is at least necessary to use two different antibodies.

On the other hand, there is no common general knowledge as of the filing that two different monoclonal antibodies were only necessary to form a sandwich complex between an antibody and an antigen, nor can it be directly inferred that the formation of a sandwich complex is only necessary to detect Mycoplasma pneumoniae in a patient's sample. Taking the description of cited reference 2 into account, it is necessary to use a combination of proper antibodies in a case of implementing the detection on the basis of the formation of a sandwich complex by use of monoclonal antibodies.

Accordingly, when a consideration is given to the description of cited reference 1 with regard to a combination of a first monoclonal antibody and a second monoclonal antibody, cited reference 1 is silent about a combination of a specific monoclonal antibody with regard to two antibodies for the use in a lateral flow device. Further, there is no evidence showing that a specific combination of monoclonal antibodies capable of forming a sandwich complex such as a lateral flow device was well-known as of the filing.

Subsequently, a sole example of a specific immunochromatography (ICT) device described in cited reference 1 of Example 4 used anti-rCARDS antibody, and did not use an antibody against P1 protein. Further, a specific description of antibody against

P1 protein in cited reference 1 is found only in Example 3. The detection of antigen in Example 3 depends on a method using a commercially-available secondary antibody of antirabbit antibody or antimouse antibody; the method is different from the formation of a sandwich complex. Therefore, a monoclonal antibody capable of forming a sandwich complex could not be read from the description of these examples.

Furthermore, cited reference 1 describes a monoclonal antibody against monoclonal anti-genuine P1 protein antibody H136E7 and rP1 of mice, it is only H136E7 that is described as a specific monoclonal against P1 protein. Regarding a monoclonal antibody against rP1, there is no cell line that produces the monoclonal antibody, no information on amino acid sequence, etc. of a monoclonal antibody, and no information that gives a clue to the formation or non-formation of a sandwich complex with H136E7. In order to produce a lateral flow device on the basis of such description of cited reference 1, it is necessary to use H136E7 as one of monoclonal antibodies, and to use another monoclonal antibody capable of forming a sandwich complex with H136E7. Cited reference 1 fails to give a clue to the structure of such monoclonal antibody. It is thus required to obtain monoclonal antibodies in some way, investigate whether these monoclonal antibodies may form a sandwich complex with H136E7, and discover a monoclonal antibody that can produce a lateral flow device for the detection of Mycoplasma pneumoniae in a patient's sample in combination with H136E7, by trial and error.

Taking the above into account, even if the technique to obtain various monoclonal antibodies was a matter of well-known art, it cannot be said that the lateral flow device of cited invention 1 as the revocation decision finds may be produced directly on the basis of the description of cited reference 1 and the common general knowledge as of the filing.

Therefore, it cannot be said that cited reference 1 describes (or substantially describes) the cited invention.

B A consideration is given to the detection from a patient's sample (clinical sample).

Cited reference 1 describes the detection of Mycoplasma pneumoniae in a patient's sample from a patient's sample in Example 7. This method is based on antigen-capture EIA with CARDS as an antigen for detection. Thus this is different in antigen and detection method from the device of the cited invention 1 that detects P1 protein on the basis of the formation of a sandwich complex. Moreover, it cannot be said that the description of Example 7 of cited reference 1 shows that the detection of Mycoplasma pneumoniae from a patient's sample (clinical sample) was possible.

Also from this viewpoint, it cannot be said that cited reference 1 describes (or

substantially describes) the cited invention.

(3) As aforementioned, in determining the inventive step, the revocation decision overlooked the different features with respect to the use of monoclonal antibodies as a first antibody and a second antibody and with respect to the detection of Mycoplasma pneumoniae in a patient's sample as a consequence of making an error in the finding of the cited invention, and it reached a conclusion of lack of inventive step without making a determination of whether these different features were easily conceivable, and revoked the patent (for this reason). Therefore, the errors in the finding of the cited invention and the overlooking of the different features affect the conclusion of the revocation decision.

Therefore, grounds 1 for rescission as Plaintiff argues have a point as far as the above discussion goes. Without considering the remaining grounds for rescission, the revocation decision should be rescinded.

Judgment rendered on November 6, 2018
2017 (Gyo-Ke) 10117 A case of seeking rescission of patent revocation decision
Date of conclusion of oral argument: August 23, 2018

Judgment

Plaintiff: Alfresa Pharma Corporation

Defendant: Commissioner of the Japan Patent Office

Main text

1 A decision of revocation in connection with Opposition No. 2016-700611 that Japan Patent Office made on April 18, 2017 shall be rescinded.

2 The court costs shall be borne by Defendant.

Facts and reasons

No. 1 Claim

The same as Main text

No. 2 Summary of the case

1 Outline of procedures, etc. at the JPO

(1) Plaintiff is a patentee of Patent No. 5845033 (a patent application on September 26, 2011, registered on November 27, 2015, the number of claims: 8, hereinafter referred to as "the Patent"), titled "IMMUNOCHROMATOGRAPHY TEST DEVICE AND KIT FOR MYCOPLASMA PNEUMONIAE DETECTION."

(2) An opposition to the grant of the Patent was filed by P, Q, R, and S that are not the parties to the case (Opposition No. 2016-700611), in the proceeding for the motion, a reason for revocation was notified on September 16, 2016, a written opinion was submitted on November 22, 2016, a notice of reasons for revocation was made on January 19, 2017 (Preliminary notice of the decision), and a written opinion and a request for correction were submitted on March 27, 2017 (hereinafter referred to as "Correction").

(3) The Japan Patent Office made a decision on April 18, 2017 for the Opposition to the grand of the Patent to the effect that "The scope of claims of Patent No. 5845033 may be corrected in accordance with Claims [1-6] and [7, 8] after the correction as in the corrected scope of claims attached to the written correction request. Patents according to Claims 1 to 8 of Patent No. 5845033 should be revoked." (hereinafter referred to as "the revocation decision") and its certified copies were served for Plaintiff on April 28.

(4) Plaintiff, who was against the revocation decision, filed a suit seeking for the

rescission of the decision on May 26, 2017.

2 The statement of the Claims

The statement of the scope of the claims after the Correction is set forth as below (An underline denotes a corrected part. The constituent elements of Claim 1 are divided by the revocation decision. Hereinafter, collectively referred to as "the patent invention," and when individually identified, identified as "patent invention 1," etc. in accordance with a number of a claim. Further, the specification and the drawings according to the patent invention [Exhibit Ko 2] are collectively referred to as "the specification").

[Claim 1]

(A) (A-1) as an antibody in an immunochromatography test device and a detection kit,

(A-2) comprising a monoclonal antibody specific to P1 protein antigen derived from Mycoplasma pneumoniae

(A-3) An immunochromatography test device for the detection of Mycoplasma pneumoniae infection from a sample,

(B) the device comprising a first monoclonal antibody and a second monoclonal antibody different from the first monoclonal antibody, and

(C) a membrane carrier,

(D) wherein said first monoclonal antibody is fixed with the membrane carrier to configure a detection site,

(E) wherein said second monoclonal antibody is (E-1) labeled with a label substance and (E-2) disposed at a position distant from the detection site so as to allow movement in the membrane carrier,

(F) (F-1) wherein the sample excluding a concentrate has (F-2) means for causing a Mycoplasma pneumoniae antigen and the second monoclonal antibody labeled with the label substance to bind together in a label support member to form a complex in a case where the Mycoplasma pneumoniae antigen is present,

(G) means for causing the complex to deploy via the membrane carrier, bind with the first monoclonal antibody fixed in the detection site, and aggregate to produce a color.

(H) An immunochromatography test device for the detection of Mycoplasma pneumoniae infection

[Claim 2]

The immunochromatography test device of Claim 1, wherein said label substance is an insoluble particulate substance.

[Claim 3]

The immunochromatography test device of Claim 2, wherein said insoluble

particulate substance is a colored synthetic polymer particle or a metal colloid particle.

[Claim 4]

The immunochromatography test device of any one of Claims 1 to 3, wherein said immunochromatography test device is intended for the diagnosis of Mycoplasma pneumoniae infection, using a sample of a biological material or a pretreated product excluding its concentrate.

[Claim 5]

The immunochromatography test device of Claim 4, wherein said biological material is a pharynx swab or nasal cavity aspirate.

[Claim 6]

A kit for the detection of Mycoplasma pneumoniae comprising the immunochromatography test device of any one of Claims 1 to 5.

[Claim 7]

A method for detecting Mycoplasma pneumoniae infection from a sample by detecting Mycoplasma pneumoniae P1 protein,

in an immunochromatography test device, having a first monoclonal antibody that is capable of specifically binding to the P1 protein derived from Mycoplasma pneumoniae and is fixed with a detection site of a membrane carrier and a second monoclonal antibody different from the first monoclonal antibody that is capable of specifically binding to the P1 protein derived from the Mycoplasma pneumoniae and is labeled with a label substance and supported by a label support member, as an antibody in an immunochromatography test device and a detection kit, wherein one or both of said first monoclonal antibody and said second monoclonal antibody are monoclonal antibodies capable of specifically binding to the P1 protein,

the method comprising the steps of: adding the sample excluding a concentrate to a sample additive member of the immunochromatography test device;

causing a protein derived from the Mycoplasma pneumoniae and the second monoclonal antibody labeled with the label substance to bind together in a label support member to form a complex in a case where the Mycoplasma pneumoniae is present in the sample; and

causing the complex to deploy via the membrane carrier, bind with the first monoclonal antibody fixed in the detection site, and aggregate to produce a color.

[Claim 8]

The method for the detection of Mycoplasma pneumoniae infection of Claim 7, wherein said label substance is an insoluble particulate substance.

3 Gist of reasons of the revocation decision

(1) The reasons for the revocation decision are as per the attached copy of the written revocation decision. In summary, the patent inventions were easily conceivable by a person ordinary skilled in the art on the basis of the invention described in the following cited reference 1 (Cited invention 1) and well-known matters in the technical field, and were granted in violation of the provision of Article 29, paragraph (2) of the Patent Act, and thus should be revoked.

Note that the publications cited by the revocation decision are set forth as below:

Cited reference 1: International Publication No. 2008/021862 (Exhibit Ko 4, Otsu 1)

Cited reference 2: Rapid Diagnosis of Mycoplasmas, Edited by I. Kahane and A. Adoni, Plenum Press, New York, 1993, P 195-205 (Exhibit Ko 5, Otsu 8)

Cited reference 3: Japanese Unexamined Patent Application Publication No. 1993-304990 (Exhibit Ko 6, Otsu 9)

Cited reference 4: Journal of General Microbiology (1992), 138, 407-422 (Exhibit Ko 7, Otsu 10)

Publication A: Japanese Unexamined Patent Application Publication No. 2001-33457 (Exhibit Ko 8)

Publication B: National Publication of International Patent Application No. 2005-506342 (Exhibit Ko 9)

Publication C: Japanese Unexamined Patent Application Publication No. 1987-206447 (Exhibit Ko 10)

Cited reference D: Japanese Unexamined Patent Application Publication No. 2009-162558 (Exhibit Ko 11)

("Cited reference D" is recognized as a typographical error of "Publication D".)

(2) Cited invention 1 as the revocation decision found, and the identical features and the different features between Cited invention 1 and the Invention 1 are set forth below:

A Cited invention 1

(a) A lateral flow device comprising an isolated antibody specifically binding to an epitope derived from M. Pneumoniae protein P1,

(b) comprising a porous test strip including at least one site including the antibody,

(c) wherein said lateral flow device includes a membrane as a carrier, on which

(c-i) a sample receiving site including the first antibody that is specific to a polypeptide epitope of P1 of M. Pneumoniae and labeled with gold colloid so as to allow it to move; and

(c-ii) a capture site disposed on a carrier, in which a second antibody specific to a polypeptide epitope of P1 of M. Pneumoniae,

are disposed:
(d) wherein the union of [Labeled first antibody] - [Polypeptide of P1 of M. Pneumoniae] - [Second antibody fixed with a capture site on a membrane as a carrier] is formed,
(e) wherein a signal generation with a label of gold colloid conjugated with the first antibody specific to a polypeptide epitope of P1 of M. Pneumoniae, which is localized in the capture site of the membrane as a carrier means the presence of M. Pneumoniae in a patient's sample, and
(f) wherein said first antibody and said second antibody are monoclonal antibodies.
(h) A lateral flow device
B Identical features
(A) (A-1) as an antibody in an immunochromatography test device and a detection kit,
(A-2) comprising a monoclonal antibody specific to P1 protein antigen derived from Mycoplasma pneumoniae
(A-3) An immunochromatography test device for the detection of Mycoplasma pneumoniae infection from a sample,
(B) the device comprising a first monoclonal antibody and a second monoclonal antibody different from the first monoclonal antibody, and
(C) a membrane carrier,
(D) wherein said first monoclonal antibody is fixed with the membrane carrier to configure a detection site,
(E) wherein said second monoclonal antibody is (E-1) labeled with a label substance and
(E-2) disposed at a position distant from the detection site so as to allow movement in the membrane carrier,
(F) (F-1') wherein the sample has (F-2) means for causing a Mycoplasma pneumoniae antigen and the second monoclonal antibody labeled with the label substance to bind together in a label support member to form a complex in a case where the Mycoplasma pneumoniae antigen is present,
(G) means for causing the complex to deploy via the membrane carrier, bind with the first monoclonal antibody fixed in the detection site, and aggregate to produce a color.
(H') An immunochromatography test device
C Different features
A sample of (F-1') and "immunochromatography test device" of (H') are indefinite as to whether cited invention 1 comprises a "concentrate" in a patient's sample. Cited reference 1 lacks examples, and it is indefinite as to whether a lateral flow device may

be used as "for the detection of Mycoplasma pneumoniae infection" also in a patient's sample that has not been concentrated, whereas the immunochromatography test device of patent invention 1 is "for the detection of Mycoplasma pneumoniae infection" even if a sample should "exclude a concentrate."

4 Grounds for rescission

(1) Errors in the finding of cited invention as well as the identical features and the different features (Grounds 1 for rescission)

(2) Errors in the determination of the different features (Grounds 2 for rescission)

(3) Errors in the finding of significant function and effect (Grounds 3 for rescission)

(omitted)

No. 4 Court decision

1 Regarding the patent invention

(1) The specification generally has the following descriptions (Exhibit Ko 2):

A Technical Field

[0001] The present invention relates to an immunochromatography device and a kit for the detection of Mycoplasma pneumoniae.

B Background Art

[0002] Apart from typical bacterial pneumonia, the cause of abacterial pneumonia (atypical pneumonia) exhibiting a pulmonary infiltrate image in chest roentgenogram is Mycoplasma pneumoniae by 30 to 40% (60% in an epidemic period). Mycoplasma isolated from human is of 7 kinds. The one which has a clear evidence of pathogenicity is Mycoplasma pneumoniae. ...

[0005] For diagnosis of Mycoplasma pneumoniae infection, various methods are utilized as set forth below.

[0006] A method for isolating and cultivating a sample such as pharynx swab and spitting is used for confirmed diagnosis. It requires a special medium and a long period (2 to 4 weeks), and non-testable cases arise by 5 to 10%.

[0007] A method for detecting an antibody due to Mycoplasma pneumoniae infection detects a serum antibody by use of, e.g., particle agglutination assay (PA), hemagglutination test (IHA), and complement-fixation test (CF). ... The method also includes the detection method of Mycoplasma antibodies IgM, IgG, and IgA by use of enzyme-linked immunoassay (ELISA).

[0008] In a detecting method of Mycoplasma pneumoniae nucleic acid in a sample such as pharynx swab and spitting by use of gene amplification technique, the

determination by use of polymerase chaining reaction (PCR) is closely correlated with a result of isolation culture method as confirmed diagnosis. However, PCR involves complicated operation, and requires special equipment, and takes several hours for measurement, and is not familiar to doctors in private practice. Further, the utilization of LAMP method (Loop-Mediated Isothermal Amplification) is also reported as a method easier than PCR ... .

[0009] Incidentally, there are reports of a monoclonal antibody specific to P1 protein of Mycoplasma pneumoniae (also known as an adhesive protein or 168 kd protein)... and a monoclonal antibody specific to Ribosomal Protein L7/L12 protein of Mycoplasma pneumoniae. Further, a monoclonal antibody specific to the glycero-type glycolipid antigen produced by Mycoplasma pneumoniae is also reported. ...

[0010] There is a strong need in the medical front to determine the presence or absence of Mycoplasma pneumoniae infection in an initial stage of infection for the selection of antibiotics. Immunochromatography is easy in operation without the need for special equipment, allowing for measurements within several tens of minutes. Also in Mycoplasma pneumoniae infection, similarly to the other infections, there is a need for an immunochromatography that is kit easily usable in the medical front and rapidly measuring an antigen; however, such kit was difficult to realize due to a smaller amount of antigen compared to the other infections.

C Problem to be solved by the invention

[0012] The objective of the present invention is to provide a kit capable of easily and rapidly detecting Mycoplasma pneumoniae infection.

D Means for solving the problem

[0013] The present invention provides an immunochromatography test device for the detection of Mycoplasma pneumoniae, comprising an antibody specific to a protein antigen derived from Mycoplasma pneumoniae.

[0014] In one embodiment, the above protein antigen derived from Mycoplasma pneumoniae is a Mycoplasma pneumoniae P1 protein.

[0015] In one embodiment, the above immunochromatography test device comprises a first antibody and a second antibody specific to the protein antigen derived from the above Mycoplasma pneumoniae, and a membrane carrier, wherein said first antibody is fixed with the membrane carrier to configure a detection site, and said second antibody is labeled with a label substance, and disposed at a position distant from the detection site so as to allow movement in the membrane carrier.

[0016] In one embodiment, the above label substance is an insoluble particulate substance.

[0017] In a further embodiment, the above insoluble particulate substance is a colored synthetic polymer particle or a metal colloid particle.

[0018] In a further embodiment, the above immunochromatography test device is for diagnosis of Mycoplasma pneumoniae infection using a sample of a biological material or its pretreated product.

[0019] In a further embodiment, the above biological material is a pharynx swab or nasal cavity aspirate.

[0020] The present invention further provides a kit for the detection of Mycoplasma pneumoniae comprising the above immunochromatography test device.

E Effects of the Invention

[0021] According to the present invention, there is provided an immunochromatography test device and a kit capable of easily and rapidly detecting Mycoplasma pneumoniae.

F Embodiments for carrying out the invention

[0022] (Antibody)

An antibody specific to a protein antigen derived from Mycoplasma pneumoniae, e.g. an antibody specific to Mycoplasma pneumoniae P1 protein (hereinafter simply referred to as "anti-Mycoplasma pneumoniae P1 protein antibody"), is used as an antibody in immunochromatography test device and a detection kit of Mycoplasma pneumoniae of the present invention. Mycoplasma pneumoniae P1 protein is a protein necessary for adhering Mycoplasma to living cells, and is also referred to as an adherence factor, and a protein with a molecular weight of 168 kd.

[0023] An antibody specific to a protein antigen derived from Mycoplasma pneumoniae, e.g. anti-Mycoplasma pneumoniae P1 protein antibody, may be either of polyclonal antibody or monoclonal antibody, but a monoclonal antibody is preferable.

[0025] An antibody specific to a protein antigen derived from Mycoplasma pneumoniae, e.g. anti-Mycoplasma pneumoniae P1 protein monoclonal antibody, may be obtained from a hybridoma manufactured by a conventional method. ...

[0026] "Antibody" used herein includes an antibody and a protein antigen derived from Mycoplasma pneumoniae substantially equivalent to the antibody, e.g. an antibody fragment having specificity to P1 protein of Mycoplasma pneumoniae and a modified antibody. ...

[0029] (Immunochromatography test device and detection kit)

"Immunochromatography test device" used herein is a device configured to allow an antigen, a labeled antibody, and a capture antibody fixed on a membrane carrier to form a complex by antigen-antibody reaction when a sample is moving on the

membrane carrier (also referred to as "deploying") by capillary action and detect the formation of the complex via a label. "Labeled antibody" used herein means an antibody labeled with the above label substance, and "capture antibody" means an antibody that is fixed on a membrane carrier to capture a complex between an antigen and a labeled antibody in the deployed sample ("sample antigen-labeled antibody complex") by binding to an antigen of the sample antigen-labeled antibody complex. "Membrane carrier" means a membrane that causes a sample, a sample antigen-labeled antibody complex, a labeled antibody, etc. to move by capillary action (i.e. "deploying").

[0030] The "immunochromatography test device" of the present invention comprises an antibody specific to a protein antigen derived from Mycoplasma pneumoniae, e.g. anti-Mycoplasma pneumoniae P1 protein antibody. ...

[0031] The term "fix" used herein means that an antibody is disposed so as not to allow movement. The term "support" means that an antibody is disposed so as to allow movement. "Labeled antibody" is an antibody labeled with a label substance with specificity to a protein antigen derived from the above Mycoplasma pneumoniae, e.g. anti-Mycoplasma pneumoniae P1 protein antibody, and further "capture antibody" is an antibody against a protein antigen derived from Mycoplasma pneumoniae, e.g. anti-Mycoplasma pneumoniae P1 protein antibody, which is an antibody for capturing the antigen with a state being fixed with a membrane carrier. "Labeled antibody" and "capture antibody" are preferably antibodies with different specificities to a protein antigen derived from Mycoplasma pneumoniae, e.g. individual different anti-Mycoplasma pneumoniae P1 protein antibodies. ... An example of a combination of anti-Mycoplasma pneumoniae P1 protein antibodies used as "labeled antibody" and "capture antibody" is shown in the following Example 1. Note that a combination of these antibodies may be the opposite, and is not limited to the combination.

[0032] Immunochromatography test device preferably comprises a first antibody, a second antibody, and a membrane carrier. Both a first antibody and a second antibody are specific to a protein antigen derived from Mycoplasma pneumoniae, e.g. Mycoplasma pneumoniae P1 protein, wherein said first antibody is fixed with the membrane carrier to configure a detection site, and said second antibody is labeled with a label substance, and disposed at a position distant from the detection site so as to allow movement in the membrane carrier. The first antibody and the second antibody are preferably antibodies with different specificities to a protein antigen derived from Mycoplasma pneumoniae, e.g. individual different anti-Mycoplasma pneumoniae P1 protein antibodies. The first antibody corresponds to the above

"capture antibody," and the second antibody corresponds to the above "labeled antibody." "Detection site" is a site to capture a sample antigen-labeled antibody complex by a capture antibody of the first antibody fixed on a membrane carrier and detect the presence of antigen.

[0034] A label support member supporting the second antibody ("labeled antibody") is disposed upstream of the detection site of the membrane carrier. The label support member and the membrane carrier may or may not be in contact with each other. It is preferable that the bottom surface of the downstream region in the labeled support member and the upper surface of a region (preferably an edge part) in the upstream of the detection site of the membrane carrier are in contact with each other. The sample additive member is disposed upstream of the label support member, preferably the bottom surface in at least a downstream region of the sample additive member is in contact with the upper surface in an upstream region of the label support member. A part of the label support member is preferably sandwiched between the bottom surface of the sample additive member and the upper surface in an upstream region of the membrane carrier. An absorption member is disposed downstream of the membrane carrier, so that at least the bottom surface of an upstream region in the absorption member may be in contact with the upper surface in a downstream region of a detection site in the membrane carrier.

[0035] The terms "upstream" and "downstream" used herein mean relative directions on the premise that a side from which a sample is added is an upstream side, whereas a side of the sample moving and deploying (flowing) is a downstream side in a longitudinal direction of the immunochromatography test device. In the specification, a detection surface of the device (i.e. a surface on which a capture antibody is fixed) is an "upper surface," whereas the opposite surface is a "bottom surface."

[0036] Sample additive member receives a sample and causes the sample to be uniformly distributed in a device. Materials of sample additive member may include cotton, glass fiber, porous synthetic resin such as porous polyethylene and porous polypropylene, and cellulose fiber. Sample additive member may be woven fabric, nonwoven fabric, paper filter, sheet, and film, etc. made of these materials. The sample additive member is also referred to as a sample pad.

[0037] The label support member supports a label antibody in a dried state, and a label antibody is released when immersed into a liquid. Materials of the label support member may include glass fiber, cellulose fiber, plastic (e.g. polyester, polypropylene, polyethylene etc.) fibers, etc. The label support member is immersed into a proper buffer solution comprising a labeled antibody, or a proper buffer solution comprising a

labeled antibody is added to the label support member, and subjected to drying to support the labeled antibody. ...

[0039] The aforementioned antibody that is specific to a protein antigen derived from Mycoplasma pneumoniae and labeled with a label substance, e.g. a labeled anti-Mycoplasma pneumoniae P1 protein antibody itself, may be utilized as a label substance for control. In this case, an antibody that is specific to a protein antigen derived from Mycoplasma pneumoniae and labeled with a label substance, e.g. an antibody capable of binding to a labeled anti-Mycoplasma pneumoniae P1 protein antibody itself (anti-rabbit IgG antibody, anti-mouse IgG antibody), may be utilized as a substance capable of binding with a label substance for control.

[0040] When a liquid sample added to a device (preferably via a sample additive member) invades a label support member, a label antibody supported by a label support member is released, and if the sample includes Mycoplasma pneumoniae antigen (P1 protein), the sample antigen and the label antibody forms a complex ("sample antigen-label antibody complex") by antigen-antibody reaction in the label support member. Subsequently, a label antibody liberated without bonding to sample, sample antigen-label antibody complex, or sample antigen moves toward the upstream of the membrane carrier from the label support member, and subsequently moves in a membrane carrier; i.e., may deploy toward downstream.

[0042] Membrane carrier has a detection site on which a capture antibody is fixed. It is preferable that membrane carrier further has a control site in which a substance capable of bonding to a label substance for control is fixed to confirm whether or not a sample has been appropriately deployed. The substance capable of bonding to a label substance for control is as mentioned above regarding the label substance for control.

[0043] The detection site and, if necessary, the control site may be disposed linearly in a traverse direction of a deploying direction on a membrane carrier (also referred to as "test line" and "control line," respectively). The positions of a detection site and a control site on a membrane carrier are not limited, but the control site is usually disposed in the downstream of the detection site.

[0045] Once a sample, a sample antigen-label antibody complex, and a label substance for control (preferably a labeled antibody that does not bind to a sample antigen), etc. reach upstream of the membrane carrier, these are deployed downstream. A capture antibody fixed with a detection site captures a sample antigen-labeled antibody complex and allows for the detection via a label. If a membrane carrier has a control site, a label passing through a detection site (preferably a labeled antibody that does not bind to a sample antigen) is captured by a substance capable of binding to a label

substance for control fixed with the control site.

[0046] Absorption member absorbs a sample deployed on a membrane carrier in an immunochromatography test device. The absorption member is usually disposed in contact with an upper surface of the membrane carrier so as to overlap with a downstream region of the control site if it is present downstream of the detection site of the membrane carrier. The immunochromatography test device equipped with an absorption member at such a position allows the deploying speed of the sample to be accelerated. ...

[0051] In the immunochromatography test device of the present invention, once a sample pretreated as necessary is dropped onto a sample additive member, the sample invades a label support member and releases a labeled antibody and causes a mixture of a sample and a labeled antibody to move on a membrane carrier, and deploy toward the detection site on a membrane carrier. If a sample contains a Mycoplasma pneumoniae antigen, the sample antigen and a labeled antibody forms a complex of sample antigen-labeled antibody, and then the complex is captured at the detection site by a capture antibody due to an antigen-antibody reaction, and aggregates the complex to produce a color. Therefore, a degree of coloring in a detection site may be observed by sight, and the presence or the absence of antigen in a sample may be determined. Further, if a membrane carrier has a control site, a label substance for control released from a label support member is captured by a substance capable of binding to a label substance for control of a control site, and aggregated to produce a color. In a case that a labeled antibody is also used as a label substance for control, the labeled antibody remaining without forming a complex in a label support member passes through a detection site, and is captured by a substance capable of binding to the labeled antibody fixed on a downstream control site and aggregated to produce a color.

[0054] A sample to be tested with an immunochromatography test device of the present invention is a sample possibly including Mycoplasma pneumoniae. The immunochromatography test device of the present invention may be used for diagnosis of Mycoplasma pneumoniae infection. A sample may include, for example, biological materials sampled from a sample suspected of Mycoplasma pneumoniae infection and a material obtained by the pretreatment of the biological materials. Biological materials may include pharynx swab, nasal cavity swab, nasal cavity aspirate, nasal cavity rinsing, spitting, alveolar lavage, rectal swab, ... in particular, pharynx swab, nasal cavity swab, nasal cavity aspirate, nasal cavity rinsing and spitting are preferable, and pharynx swab and nasal cavity aspirate are further preferable. ...

[0056] A device for sampling a sample may include cotton swab, swab, platinum loop,

dropper, or a spoon-shaped device. Particularly in a case that a sample such as nasal cavity swab, nasal cavity aspirate, pharynx swab, alveolar lavage, and spitting are sampled, cotton swab and swab are used as a sampler.

[0057] A pretreatment of a sample may include dissolving a test sample sampled from an object under study suspected of Mycoplasma pneumoniae infection into an agent for pretreatment to prepare a liquid for dropping into an immunochromatography test device. Such pretreatment makes a sample a deployable liquid. This agent for pretreatment in the specification is also referred to as "sample extraction liquid." The sample extraction liquid may usually include a buffer for maintaining a salt concentration and pH to certain levels. For a buffer, a buffer commonly used in an immunological test may be used, which includes Tris buffer, phosphate buffer, Good's buffer, etc. Furthermore, a sample extraction liquid may contain a surfactant for the purpose of decreasing a non-specific reaction to the extent that does not interfere with a specific aggregation reaction. The surfactant may include Triton X-100 (product name): polyethyleneglycol mono-p-isooctylphenyl ether, Tween 20: polyoxyethylene sorbitan monolaurate, Tween 80: polyoxyethylene sorbitan monooleate, NonidetP-40, ZWITTERGENT 3-14: ..., CHAPS: 3-[(3-Cholamidopropyl)dimethylammonio] propanesulfonic acid ... etc., or a mixture of two or more thereof. The sample extraction liquid may contain proteins such as bovine serum albumen, immune globulin and casein, serums of rabbit or mouse for the purpose of decreasing a non-specific reaction.

G Examples

[0060] (Example 1: Manufacture of immunochromatography test device)

(1-1. Preparation of label support member)

An anti-Mycoplasma pneumoniae P1 protein monoclonal antibody ... was diluted with 5 mM phosphate buffer (pH 7.4) in a concentration of 0.05 mg/mL. To 0.5 mL of an gold colloid suspension (... average particle size 60 nm) there was added 0.1 mL of 50 mM phosphate buffer (pH 7.4), and they were mixed together, followed by a further addition of the above diluted 0.1 mL solution of anti-Mycoplasma pneumoniae P1 protein monoclonal antibody, and left to stand for 10 minutes at a room temperature. To a solution after still standing there was added 0.1 mL of 10 mass% bovine serum albumin (BSA) solution diluted with 10 mM phosphate buffer, which was sufficiently stirred, and then subjected to a centrifugal separation for 15 minutes at 8000*g. After removal of a supernatant, 1 mL of 10 mM phosphate buffer at a pH of 7.4 was added to a residue, and a colloid was well-dispersed with a sonicator, and then subjected to a centrifugal separation for 15 minutes at 8000*g. Again, removing a supernatant, 10

mM phosphate buffer at a pH of 7.4 was added to a residue and well-dispersed with a sonicator to obtain a labeled antibody solution. This labeled antibody solution was uniformly added to a pad with a width of 16 mm and a length of 100 mm made of glass fiber, and then dried with a vacuum drier to obtain a label support member.

[0061] (1-2. Manufacture of nitrocellulose membrane carrier comprising a detection site and a control site)

An anti-Mycoplasma pneumoniae P1 protein monoclonal antibody different from the one for the manufacture of the labeled antibody solution of the above item 1-1 ... was diluted with a phosphate buffer containing 5 mass% isopropylalcohol (pH7.4) at a concentration of 1.3 mg/mL to prepare an antibody solution for the fixation of detection site. This antibody solution for the fixation of detection site was linearly coated in a coating amount of 1 μL/cm ... at a position 1 cm distant from one end of a longitudinal axis (this end is set to an upstream end of the deploying direction, whereas the opposite end was set to an end in a downstream side)... of a nitrocellulose membrane with a length of 25 cm x a width of 2.5 cm. Furthermore, at a position 1.5 cm distant from the upstream end of a nitrocellulose membrane there was linearly coated an antibody solution for the fixation of control site in which anti-mouse IgG antibody was diluted in a concentration of 1 mg/mL ... in a coating amount of 1 μL/cm. After coating, the solution was dried for 60 minutes at 42°C to obtain a nitrocellulose membrane carrier comprising a detection site and a control site.

[0062] (1-3. Manufacture of immunochromatography test device)

A backing sheet made of a plastic was adhered to a side (supposing that this surface to be a bottom surface) opposite to a surface (supposing that this surface to be an upper surface) of coating an antibody in a nitrocellulose membrane carrier of the above item 1-2. Subsequently, the labeled support member of the above item 1-1 was disposed on and attached to an upper surface of the above nitrocellulose membrane so that an upstream end of the nitrocellulose membrane overlapped by 2 mm, and furthermore, a sample pad made of grass fiber with a width of 5 mm x a length of 23 mm ... was disposed on and attached to an upper surface of the label support member overlapped by 2 mm. Furthermore, an absorption pad with a width of 5 mm x a length of 25 mm ... was attached to the upper surface of the above nitrocellulose membrane carrier so that a downstream end of the nitrocellulose membrane carrier might overlap by 15 mm. Finally, the laminate was cut out by 5 mm each in a longitudinal direction to obtain an immunochromatography test device.

[0063] (Example 2: Detection of Mycoplasma pneumoniae from a nasal cavity aspirate sample)

A nasal cavity aspirate was sampled by a conventional method from patients clinically suspected of Mycoplasma pneumoniae infection. A part of each sample of the sampled nasal cavity aspirate served for the detection of Mycoplasma pneumoniae using the immunochromatography testing device ("Immunochromatography Determination").

[0064] A sterilized cotton swab was immersed into a nasal cavity aspirate and taken out, and then immersed into a sample extraction liquid dispensed into a tube, and stirred. The cotton swab was pinched from the outside of the tube, and drawn several times to squeeze the sample well, and then the cotton swab was taken out. This resulted in a sample. Subsequently, a nozzle cap with a filter membrane attached to a tube was placed. Pinching a middle part of the tube, three drops of a liquid sample (120 μL) were dropped into an immunochromatography test device of Example 1, and left to stand for 15 minutes, and then a determination was made. Note that the above cotton swab and sample extraction liquid were the ones attached to a test kit of adenovirus of "Prime Check adeno" (manufactured by Alfresa Pharma Corporation). Further, a tube and a nozzle cap with filter were the ones attached to a test kit of Influenza A and B of "Check Flu A*B" (manufactured by Alfresa Pharma Corporation).

[0065] The determination was valid in a case that a control site produced a red-purple color, and positive in a case that a detection site also produced a red-purple color. In a case that the control site produced a red-purple color, but the detection site did not produce any color, it was determined as negative. The result is shown in Table 1.

[0066] Another part of each sample of a nasal cavity aspirate from the same patient served as a sample for PCR determination. ... The result of PCR determination is additionally shown in Table 1.

[0067]

[Table 1]

Sample Number	Immunochromatography Determination	PCR
1	-	-
2	+	+
3	-	-
4	+	+
5	+	+
6	+	+

7	+	+
8	+	+
9	+	+

[0068] As shown in Table 1, the results of immunochromatography determination coincided with the results of PCR determination in every sample.

[0069] (Example 3: Detection of Mycoplasma pneumoniae from a pharynx swab sample)

Pharynx swabs were sampled by a conventional method using sterilized cotton swabs from patients clinically suspected of Mycoplasma pneumoniae infection. Except for this, in a similar manner to Example 2, an immunochromatography determination and a PCR determination were made.

The results are shown in Table 2.

[0070]

[Table 2]

Sample Number	Immunochromatography Determination	PCR
101	+	+
102	+	+
103	+	+
104	+	+
105	+	+
106	+	+
107	-	-
108	+	+
109	-	-
110	+	+
111	+	+

[0071] As shown in Table 2, the results of immunochromatography determination coincided with the results of PCR determination in every sample.

[0072] (Example 4: Study of reactivity with various microorganisms including Mycoplasma pneumoniae)

Bacterial bodies of various microorganisms including Mycoplasma pneumoniae were diluted with a sample extraction liquid at a concentration of 10^7 CFU/mL to prepare bacterial body solutions. Three drops of a liquid sample (120 μL) were dropped into

the immunochromatography test device of Example 1, and left to stand for 15 minutes, and then a determination was made. Further, a sample extraction liquid was dropped in a similar manner as a negative control to make a determination. Note that the sample extraction liquid was one attached to a test kit of adenovirus of "Prime Check adeno" (manufactured by Alfresa Pharma Corporation). The result is shown in Table 3.

[0073]

[Table 3]

Bacterial species	Bacteria concentration (cfu)	Immunochromatography Determination
Mycoplasma pneumoniae	10^7/mL	+
Moraxella catarrhalis	10^7/mL	-
Haemophilus influenzae	10^7/mL	-
Eikenella corrodens	10^7/mL	-
Streptococcus mutans	10^7/mL	-
Acinetobacter baumannii	10^7/mL	-
Candida albicans	10^7/mL	-
Neisseria gonorrhoeae	10^7/mL	-
Staphylococcus epidermidis	10^7/mL	-
Staphylococcus group F	10^7/mL	-
Staphylococcus pneumoniae	10^7/mL	-
Klebsiella pneumoniae	10^7/mL	-
Chlamydia pneumoniae	10^7/mL	-
Sample extract		-

[0074] As shown in Table 3, a bacterial body solution of Mycoplasma pneumoniae was positive by an immunochromatography determination. Bacterial body solutions and sample extraction liquids of all microorganisms but Mycoplasma pneumoniae were negative by an immunochromatography determination.

[0075] (Comparative Example 1: Manufacture of immunochromatography test device using an antibody specific to glycolipid antigen derived from Mycoplasma pneumoniae)

Except for the use of anti-Mycoplasma pneumoniae Glycolipid monoclonal antibody as an antibody for the preparation of labeled antibody solution, ... and the use of anti-Mycoplasma pneumoniae Glycolipid monoclonal antibody different from an antibody for the use in the preparation of labeled antibody solution as an antibody for the

preparation of an antibody solution for the fixation of a detection site, in compliance with a procedure of Example 1, an immunochromatography test device was manufactured.

[0076] (Example 5: Study of reactivity of anti-P1 protein antibody immunochromatography test device and anti-Glycolipid antibody immunochromatography test device in the detection of Mycoplasma pneumoniae)

Mycoplasma pneumoniae antigen ... was diluted with a sample extraction liquid in a concentration of 10 μg/mL to prepare a test liquid. Three drops of a test liquid (120 μL) were dropped into the anti-P1 protein antibody immunochromatography test device of Example 1, and left to stand for 15 minutes, and then a determination was made. Further, a sample extraction liquid was dropped in a similar manner as a negative control to make a determination. A similar operation was conducted for anti-Glycolipid antibody immunochromatography test device of Comparative Example 1. Note that the sample extraction liquid was the one attached to a test kit of adenovirus of "Prime Check adeno" (manufactured by Alfresa Pharma Corporation). The result is shown in Table 4.

[0077]

[Table 4]

	Immunochromatography Determination	
	Example 1 (Anti-P1 protein antibody)	Comparative Example 1 (Anti-Glycolipid antibody)
Sample extract liquid	-	-
Test liquid	+	-

[0078] As shown in Table 4, a sample extraction liquid was determined as negative if an anti-P1 protein antibody immunochromatography test device of Example 1 was used, whereas a test liquid was determined as positive. On the other hand, a test liquid and a sample extraction liquid were determined as negative if the anti-Glycolipid antibody immunochromatography test device of Comparative Example 1 was used.

H Industrial Applicability

[0079] According to the present invention, there are provided an immunochromatography test device and a kit capable of easily and rapidly detecting Mycoplasma pneumoniae. Immunochromatography is easy in operation without the need for special equipment, allowing for rapid measurements within several tens of

minutes. Mycoplasma pneumoniae detection by use of immunochromatography allows for easy use and rapid measurement in a medical front also in Mycoplasma pneumoniae infection where the detection in an initial stage of infection was supposed to be difficult.

(2) According to the above description, the following matters are recognized with regard to the patent invention:

The patent invention relates to an immunochromatography device and a kit for the detection of Mycoplasma pneumoniae ([0001]).

There is a strong need on the medical front to determine the presence or absence of Mycoplasma pneumoniae infection in an initial stage of infection for the selection of antibiotics. Immunochromatography is easy in operation without the need for special equipment, allowing for measurements within several tens of minutes. In Mycoplasma pneumoniae infection, similarly to the other infections, there is a need for an immunochromatography kit easily usable in the medical front and rapidly measuring an antigen; however, such kit was difficult to realize due to a smaller amount of antigen compared to the other infections ([0010]).

The patent invention provides an immunochromatography test device for the detection of Mycoplasma pneumoniae, the device comprising an antibody specific to a protein antigen derived from Mycoplasma pneumoniae ([0013]).

In one embodiment, the above protein antigen derived from Mycoplasma pneumoniae is a Mycoplasma pneumoniae P1 protein. The above immunochromatography test device comprises a first antibody and a second antibody specific to the protein antigen derived from the above Mycoplasma pneumoniae, and a membrane carrier, wherein said first antibody is fixed with the membrane carrier to configure a detection site, and said second antibody is labeled with a label substance, and disposed at a position distant from the detection site so as to allow movement in the membrane carrier ([0014], [0015]).

In a further embodiment, the above immunochromatography test device is for diagnosis of Mycoplasma pneumoniae infection using a sample of a biological material such as a pharynx swab or nasal cavity aspirate or its pretreated product ([0018], [0019]).

2 Described matters of cited reference 1

Cited reference 1 generally has the following descriptions (Exhibit Ko 4, Otsu 1. The original text is in English. A Japanese translation is basically derived from the translation submitted by Defendants, but corrected as necessary.).

(1) Background

[0002] Mycoplasma pneumoniae is an extracellular pathogen that attaches to and destroys ciliated epithelial cells of the respiratory tract mucosa. M. pneumoniae is believed to be responsible for approximately 20% to 30% of community acquired pneumonia infections and has been implicated in asthma and chronic obstructive pulmonary disease. ...

[0003] Diagnosis of the M. pneumoniae pathogen responsible in these cases is difficult and is usually based on comparison of serum antibody titers between acute and convalescent phases. A fourfold or greater rise in antibody titer in the latter compared to the former indicates that the patient was infected with the organism. Improved methods of detecting M. pneumoniae are thus needed.

(2) Summary

[001] Provided are compositions, methods, and devices for detecting M. pneumoniae. We have discovered novel epitopes of M. pneumoniae that may be used to generate antibodies and other agents capable of specifically binding the epitopes. These antibodies and other compounds may be incorporated into a variety of methods for the standardized, sensitive, and/or specific detection of M. pneumoniae.

(3) Brief Description of the Drawing(s)

[0012] FIGURE 7 depicts the sequence of a fragment of recombinant P1 ("rP1") protein (SEQ ID NO: 7). The full-length sequence of P1, an approximately 165 kDa protein, may be found at GenBank Accession Nos. AAK92039, AAK92040, AAK92038, AAK92037, NP_109829, and AAB95661. The epitopes recognized by polyclonal anti-rP1 rabbit antisera are in italics, and the epitope recognized by monoclonal H136E7 is in bold. The monoclonal antibody is described in U.S. Patent No. 4,945,041 and Kahane, et al. (1985) IAI 50:944. Only two overlapping peptides were recognized by the monoclonal antibody H136E7. The sequence in common between these peptides is shown in underlined bold.

(4) Detailed Description

[0030] 1. Epitopes of M. pneumoniae and Agents that Bind Epitopes of M. pneumoniae

[0031] Provided are isolated and/or purified polypeptides comprising epitopes of proteins from M. pneumoniae. These polypeptides are referred to herein collectively as "polypeptide epitopes of M. pneumoniae." In certain embodiments, provided are isolated and/or purified polypeptides comprising epitopes of rCARDS protein (SEQ ID NO: 1) and rP1 protein (SEQ ID NO: 7) from M. pneumoniae. For example, polypeptide sequences comprising epitopes of rCARDS protein are SEQ ID NOs: 2 through 6, and polypeptide sequences comprising epitopes of rP1 protein are SEQ ID

NOs: 8 through 10. Additional epitopes may be identified using the procedures outlined in the Exemplification for other M. pneumoniae proteins, as well as using methods for epitope identification as known to those of skilled in the art. ...

[0047] 2. Methods of Using Agents that Bind Epitopes of M. pneumoniae to Detect M. pneumoniae

[0048] Provided also are methods comprising the use of one or more antibodies, antibody fragments, or other agents specific for a polypeptide epitope of an M. pneumoniae to detect M. pneumoniae bacteria, e.g., that may have infected a subject. The antibodies, antibody fragments, or other agents may bind M. pneumoniae proteins in a sample prepared from lysed bacteria, or M. pneumoniae proteins present on M. pneumoniae bacteria.

[0049] In one embodiment, a method for detection of M. pneumoniae bacteria comprises the steps of providing a sample, preferably a respiratory sample, suspected of containing M. pneumoniae bacteria and/or proteins. A sample can be tested by any known methods in order to confirm the presence of M. pneumoniae bacteria and/or proteins in the sample. ... For example, a sample suspected of containing M. pneumoniae bacteria and/or proteins can be tested by using an immunochromatographic test device capable of detecting M. pneumoniae bacteria and/or proteins.

[0050] In certain embodiments, the sample may be a respiratory sample, such as a swab. The swab may be directly used in an assay method or device, or washed with an elution buffer and the eluate used in the assay method or device.

[0051] In embodiments, the sample is a throat swab, or is prepared therefrom. A sample may be prepared from a throat swab as follows. A throat swab is taken from a patient presenting with respiratory symptoms consistent with M. pneumoniae infection. The throat swab may be then placed in a buffer, for example, comprising a detergent such as Brij 58, Zwittergen 3-14, or cholate. Preferably, where a detergent is used, Zwittergen 3-14 is used. If appropriate, insoluble components may be removed from the sample, and the supernatant used as the test sample.

[0052] In certain embodiments, the sample may comprise M. pneumoniae proteins extracted from M. pneumoniae bacteria. Methods for bacterial cell lysis and subsequent protein extraction are well-known in the art.

[0053] Generally, the methods may include providing an antibody, antibody fragment, or other agent specific for a polypeptide epitope of M. pneumoniae, wherein the antibody, antibody fragment, or other agent binds to the epitope as present on the M. pneumoniae protein from which the polypeptide epitope was derived, if present in the

sample being tested, to form complexes. In the event that the sample does not contain the epitope, no complex is formed.

[0055] The methods may further comprise contacting a sample potentially containing complexes with a secondary antibody, antibody fragment, or other agent specific for a polypeptide epitope of M. pneumoniae. The secondary antibody, antibody fragment, or other agent may be the same as or different from the first antibody, antibody fragment, or other agent specific for a polypeptide epitope of M. pneumoniae. The secondary antibody or antibody fragment or agent may be labeled for ease of detection, and the label can be any suitable label that is able to be detected. By way of example, the label may be a radiolabel, fluorescent label, colloidal label, enzymatic label, particulate label, or molecule that is readily detectable either by its presence or activity.

[0056] The methods may further comprise detecting the presence or absence of the first antibody-M. pneumoniae protein or bacteria-second antibody complexes,. ... For example, the sample can be tested by a general immuno chromatographic test device.

[0057] In one embodiment, a method comprises: (a) contacting a sample with a first antibody, antibody fragment, or agent specific for a polypeptide epitope of M. pneumoniae to form, if M. pneumoniae protein comprising the epitope or M. pneumoniae bacterium is present in the sample, a first complex comprising the first antibody, antibody fragment, or other agent and M. pneumoniae protein comprising the epitope or M. pneumoniae bacterium; (b) contacting the sample with a second antibody, antibody fragment, or agent specific for a polypeptide epitope of M. pneumoniae to form, if the sample comprises the first complex, a second complex comprising the first complex and a first antibody, antibody fragment, or agent specific for a polypeptide epitope of M. pneumoniae; and (c) detecting whether the second complex is formed.

[0058] In certain embodiments, detection of a M pneumoniae protein is accomplished by one of the three following specific methods: (1) direct enzyme immunoassay ("EIA"); (2) Western blot; and (3) lateral flow.

[0059] For example, for the direct enzyme immunoassay, EIA plates may be coated with a first antibody, antibody fragment or other agent specific for a polypeptide epitope of M. pneumoniae and washed, then blocked and washed. A patient sample may be added to the plate, which is then incubated and washed. A secondary antibody, antibody fragment, or other agent specific for a polypeptide epitope of M. pneumoniae that is conjugated to a reporter system may be then added, and the plate incubated and washed. Substrate for the reporter system may then be added, and substrate development allowed. The development may then be stopped and the degree of substrate development read on a plate reader or other suitable device. The

presence of the polypeptide epitope of M. pneumoniae is indicated by signal development higher than reaction produced in the absence of the organism.

[0061] For use in the methods described above, kits and devices for the practice of the above-described methods are also provided. Devices for practice of the methods include lateral flow devices (wherein the reagents employed in the reaction may be dried or immobilized onto a chromatographic support contained within the device), a test strip, or other support for practice of the methods.

[0062] A method incorporating lateral flow may be practiced as follows. A patient sample may be added to the sample pad of a lateral flow device. The sample pad or other component may contain an extractant such as the detergent Zwittergen 3-14. In the device, the patient sample moves into the conjugate pad where an antibody, antibody fragment, or other agent specific for a polypeptide epitope of M. pneumoniae reacts with any protein comprising the polypeptide epitope of M. pneumoniae in the patient sample. The antibody, antibody fragment, or other agent is conjugated to a reporter (for example, gold, latex, or other microparticle, or any other type of reporter system) in a dried format. The patient sample moves through the device. If the epitope is present in the sample, the polypeptide comprising it will be captured by the antibody, antibody fragment, or other agent specific for a polypeptide epitope of M. pneumoniae localized on the membrane. Signal may be generated by the reporter on the conjugate antibody, antibody fragment, or other agent, which indicates the presence of M. pneumoniae in the patient sample. In other embodiments, the interaction of the conjugate antibody may be accomplished in a liquid format, in which case the patient sample is added to the conjugate solution.

[0063] A kit for the practice of the above methods may include a support, reagents and wash, and incubation buffers. ... Such kits and devices may have a variety of uses, including, for example, diagnosis, therapy, and other applications.

[0065] The sample application pad is a porous pad able to absorb the sample to be tested and transfer the absorbed sample to the conjugate pad by capillary action. The conjugate pad includes one or more dried labeled molecules or reagents, such as antibodies, capable of specifically binding to the one or more analytes of interest forming an analyte-labeled reagent complex. The conjugate pad may also include one or more stabilizing compounds that are able to induce thermal stability and also stability as to conditions imposed by humidity and temperature. The conjugate pad is a porous pad able to absorb the transferred sample from the sample application pad and transfer the sample to the nitrocellulose strip by capillary action. The nitrocellulose strip is able to absorb the sample from the conjugate pad and transfer the sample by

capillary action downstream to the test result zone and the control zone. The test result zone of the immunoassay device includes one or more immobilized molecules or reagents, such as antibodies or antibody fragments, capable of specifically binding to the one or more analytes of interest or any portion of the analyte-labeled reagent complex. The control zone of the immunoassay device may include one or more immobilized molecules or reagents, such as antibodies or antibody fragments, capable of specifically binding to the one or more labeled reagent. ...

[0066] When a liquid test sample is applied to the sample application pad of the device, the sample travels through the sample application pad, the conjugate pad, and nitrocellulose strip by capillary action. When the sample travels through the conjugate pad, the sample solublizes the dried labeled molecule or reagent, and if the analyte of interest is present in the sample, the solublized labeled molecule or reagent binds to the analyte of interest forming an analyte-labeled reagent complex; otherwise, if the analyte of interest is not present in the sample, no complex is formed. The analyte-labeled reagent complex in the case of a positive test, or the labeled reagent alone in the case of a negative test, then travel to the nitrocellulose strip and travel through and pass the test result zone and the control zone of the device. If the analyte of interest is present in the sample, the analyte-labeled reagent complex binds to the immobilized reagent of the test result zone forming a detectable line, and if the analyte of interest is not present in the sample, no analyte-labeled reagent complex is formed and therefore no binding occurs at the test result zone. ...

[0067] The analyte may be a protein or other component derived from M. pneumoniae or M. pneumoniae bacterium.

[0068] In one embodiment, a device may comprise a carrier upon which is disposed (a) a sample receiving zone comprising mobilizable labeled first antibodies, antibody fragments, or agents specific for a polypeptide epitope of M. pneumoniae; and (b) a capture zone comprising immobilized second antibodies, antibody fragments, or agents specific for a polypeptide epitope of M. pneumoniae. The first and second antibody, antibody fragment, or agent specific for a polypeptide epitope of M. pneumoniae may be the same, or different.

[0069] In other embodiments, a device may comprise a carrier defining a flow path extending at least from a sample receiving zone to a capture zone, mobilizable labeled first antibodies, antibody fragments, or agents specific for a polypeptide epitope of M. pneumoniae, and second antibodies, antibody fragments or agents specific for a polypeptide epitope of M. pneumoniae disposed along the flow path; wherein: a liquid sample received by the receiving zone migrates along the flow path mobilizing the

labeled antibodies, antibody fragments, or agents, and in the presence of M. pneumoniae bacteria or protein, the labeled and second antibodies, antibody fragments, or agents cooperate to capture the antibodies, antibody fragments, or agents in the capture zone.

[0070] The second antibodies, antibody fragments, or agents may be disposed in the capture zone when the device is in the unused state.

(5) Examples

[0078] Example 1: Identification of Epitopes in Recombinant CARDS

[0079] Dr. Joel Baseman and colleagues have described a toxin molecule produced by M. pneumoniae (Kannan et al, 2005, Inf. Immun. 73: 52828-34; Kannan and Baseman, 2006, PNAS 103: 6724-9). Detection of the toxin during infection might be an approach for diagnosis of the pathogen. ...

[0080] The sequence (SEQ ID NO: 1) of recombinant CARDS ("rCARDS") from M. pneumoniae is depicted in FIGURE 1. Rabbits were immunized and boosted with rCARDS protein using standard protocols. An array of overlapping peptides encompassing the rCARDS sequence was purchased. Each peptide contained 17 amino acids of the rCARDS sequence and overlapped each of its neighbor sequences by 10 amino acids. Each peptide was synthesized with a cysteine residue at its C-terminus which is used to covalently couple the peptide to commercially available microtiter plates. Peptides were bound to the microtiter plates in solvent. ...

[0081] Polyclonal anti-rCARDS rabbit antisera were subjected to a serial dilution series. A control antiserum raised against a second M. pneumoniae protein was included in the experiment. Each dilution was applied to the immobilized peptide and allowed to react. Plates were washed. Appropriate, commercially available, secondary anti-rabbit antibodies were pre-coupled to a reporter system, horseradish peroxidase, which was added in a fixed concentration and allowed to react. The plates were washed. The substrate for horseradish peroxidase was added and color development was noted. The reaction was terminated by addition of inorganic acid. The color produced was read using a microtiter plate reader. The sequences of peptides which allowed production of more color in dilution series of anti-rCARDS or anti-CARDS antibodies compared to control reaction indicated peptide epitopes. The regions of rCARDS so determined to be epitopes are indicated in italics in FIGURE 1.

[0082] Example 2: Synthesis and Evaluation of rCARDS Epitopes

[0083] Synthesis of Epitopes of rCARDS

[0084] The work in Example 1 identified epitopes in recombinant CARDS (rCARDS) using polyclonal anti-rCARDS rabbit antisera, which are potential sites for targeting

antigen capture and detection of M. pneumoniae CARDS toxin.

[0085] Peptides encompassing the identified rCARDS epitopes were synthesized (FIGURE 2). ...

[0086] Purification of Antibodies against Epitopes of rCARDS

[0087] Rabbit IgG antibodies were enriched from the rabbit anti-rCARDS polyclonal antisera of Example 1 using standard techniques. A portion was set aside and used as the starting IgG pool. Five individual columns were prepared, each containing one of the five peptides described in FIGURE 3 linked to the solid phase through the terminal cysteine. Portions of the purified IgG antibody preparation are subjected to affinity purification on each of the five immobilized peptides using standard protocols. Twenty-five to fifty micrograms of each affinity purified antibody and starting IgG preparation were conjugated to the reporter enzyme, horseradish peroxidase. The remaining affinity purified antibodies and starting IgG were utilized in a non-modified form.

[0088] Antigen capture and detection

[0089] Solid phase antigen capture and detection was performed in plastic microtiter plates. ...

[0090] Purified anti-rCARDS IgG or affinity purified anti-peptide antibodies were absorbed to the solid phase using standard methods. In some experiments, a combination of affinity purified antibodies was immobilized on the solid phase. ... Unreacted sites on the solid phase were blocked by incubation in the presence of an irrelevant protein. rCARDS protein was added to the solid phase in PBST containing irrelevant protein. A dilution series of rCARDS was simultaneously made to permit definition of the lowest detection limit of rCARDS. In some experiments, similar dilution series of a second recombinant M. pneumoniae protein (rP1) was used to challenge the specificity of the assay. Incubation allowed antigen capture by immobilized antibody. Washing was employed to remove unreacted antigen. Affinity purified anti-peptide antibodies or starting IgG pool coupled to HRP were added in individual reactions. The conjugated antibodies were allowed to bind to captured rCARDS by incubation. Washing was used to remove excess conjugate. Conjugate binding was determined with a suitable reporter substrate. The reaction was terminated by addition of inorganic acid. Antigen capture and detection were quantitated using a microtiter plate reader. The data were analyzed and plots were made. In some cases, the background reactions resulting when antigen is not included were subtracted for ease of comparison.

[0091] FIGURE 4 shows the results of all possible combinations of antigen capture

and detection using the starting IgG pool and affinity purified anti-peptide antibodies. All combinations were successful. The strongest reactions are observed using the IgG pool as both capture and detection reagents. Anti-peptide 2-HRP conjugate antibodies for detection are generally as effective as use of the IgG pool conjugate. In most cases, use of the same affinity purified antibody as capture and detection reagent is not as efficacious as use of heterologous reagents.

[0092] FIGURE 5 shows that antigen capture using the starting IgG pool, a combination of anti peptide (3, 4, and 6) antibodies and anti-peptide 4 antibodies were all equally efficacious. Anti-peptide 2 conjugate was used for detection.

[0093] Contaminating bacterial proteins produced during production of recombinant molecules are usually of concern. To exclude the possibility that the anti-rCARDS antisera shows reaction to such contaminants, the assay was challenged using a second recombinant protein, rP1 (FIGURE 6). Antigen capture is effected using the starting IgG pool; a combination of anti-peptides 3, 4, and 5; or anti-peptide 4 antibodies. Anti-peptide 2 or the IgG pool conjugate were used for detection. All possible combinations showed utility. Significant reaction with rP1 was not observed. The latter observation suggests assay specificity and indicates that antibody directed against contaminating bacterial proteins are not of concern.

[0094] Example 3: Identification of Epitopes in Recombinant P1

[0095] M. pneumoniae P1 adhesion protein has been described in the literature. Diagnosis of M. pneumoniae by detection of P1 protein may be a viable alternative to existing assays. Anti-recombinant P1 polyclonal antisera and antibodies may be useful reagents for this approach.

[0096] The sequence (SEQ ID NO: 7) of recombinant P1 ("rP1") from M. pneumoniae is depicted in FIGURE 7. Rabbits were immunized and boosted with rP1 protein using standard protocols. Samples of mouse, monoclonal anti-authentic P1 protein were obtained. An array of overlapping peptides encompassing the rP1 sequence was purchased. Each peptide contained 14 amino acids of the rP1 sequence and overlapped each of its neighbor sequences by 10 amino acids. Each peptide was synthesized with a C (cysteine, cys) residue at its C-terminus which is used to covalently couple the peptide to commercially available microtiter plates. Peptides were bound to the microtiter plates in solvent. Unreacted sites were rendered inert using a cysteine solution, and plastic, protein binding sites were blocked with a solution of bovine serum albumen. Microtiter plates were washed using standard conditions.

[0097] Polyclonal anti-rP1 rabbit antisera were subjected to a serial dilution series. A

control antiserum raised against a second M. pneumoniae protein was included in the experiment. Samples of the monoclonal antibodies were also subjected to serial dilutions; a control was an irrelevant monoclonal antibody. Each dilution was applied to the immobilized peptide and allowed to react. Plates were washed. Appropriate, commercially available, secondary antibody (anti-rabbit or anti-mouse IgG) each coupled to a reporter system, horseradish peroxidase, was added in a fixed concentration and allowed to react. Plates were washed. The substrate for horseradish peroxidase was added and color development was noted. The reaction was terminated by addition of inorganic acid. The color produced was read using a microtiter plate reader. The sequence of peptides which allowed production of more color in dilution series of anti-rP1 or anti-P1 antibodies compared to control reaction indicated peptide epitopes. Confirmation of relevant sequences was made in a second experiment; in this case, polyclonal anti-rP1 antisera were pooled prior to construction of the dilution series. The data indicate the regions shown in FIGURE 7 are epitopes. The epitope sequences are as follows:

[0098] SEQ ID NO: 8: MAFRGSWVNRLGRVESVWDLKGVWAD

[0099] SEQ ID NO: 9: EHPNALAFQVSVVE

[00100] SEQ ID NO: 10: STNSSPYLHLVKPKKVTQSDKLDDDLKNLLDPNQ

[00101] Example 4: Anti-rCARDS ICT Device

[00102] Immuno chromatography (ICT) may be more easily used in physician office laboratories than microtiter plate formats. Experiments were designed to examine the efficacy of this approach. The anti-rCARDS IgG pool was applied to two types of nitrocellulose differing in pore size at 3 concentrations. The nitrocellulose was incorporated into dipstick type devices consisting of a porous sample pad, nitrocellulose, and an absorbent pad at the distal end. Conjugates used in this work were colloidal gold coupled to the IgG pool or to antibodies that had affinity purified on SEQ ID NO: 2. In this experiment, the conjugates were maintained in a liquid state; conjugate solutions included a detergent and exogenous proteins to help prevent non-specific binding.

[00103] The strips were used in assays as follows. rCARDS was serially diluted in PBS containing BSA. rP1 was used for a specificity challenge in a subsequent experiment. Liquid conjugate was added to the sample. Strips were placed into the conjugate/sample mixture; fluid flow was initiated and allowed to proceed for 5-10 min. Next, strips were transferred to a PBST wash for 10 min. The assays were scored after 15 and 30 minutes against an arbitrary set of intensity standards (FIGURE 11). Note that a score of 3.0 is considered saturating. Scores of 0 indicate that no

reaction was seen. The data presented represent the average of two scores at 15 min. The limit of detection of rCARDS was between 0.2 and 2.0 ng/ml. Little reaction was observed with rP1, even at high levels, suggesting that rCARDS detection is specific.

[00104] Example 5: Extraction of CARDS from cultured M. pneumoniae cells

[00105] Cultured, γ-irradiated cells were used since these organisms could be safely handled on the laboratory bench without risk of infection. Gamma-irradiation is believed to preserve protein native structure.

[00106] Suitable methods of extraction were examined by adjusting aliquots of γ-irradiated M. pneumoniae to desired concentrations of the potential extraction reagent. The aliquots were subjected to micro centrifugation at 10,000-15,000 xg to pellet material not solubilized by the extraction. The supernatant from each extraction was made suitable for electrophoresis under denaturing conditions. The pellet was dispersed in PBS using the same volume as the original aliquot and was then adjusted for denaturing gel electrophoresis. A control consisted of fractionation in the absence of extraction reagent (not-treated). The supernatants and pellets were subjected to electrophoresis and were transferred to nitrocellulose via Western blotting (FIGURE 12). Nitrocellulose membranes were reacted with rabbit polyclonal-anti-rCARDS antisera followed by goat-anti rabbit IgG-horse radish conjugate. Filters were then developed with a precipitating substrate for horse radish peroxidase. An increase in intensity of the band observed in the supernatant lane compared to its paired pellet and to the untreated supernatant indicated which extraction reagent was a good candidate. Of potential extraction reagents examined, the detergents Brij 58, Zwittergen 3-14 and cholate showed efficacy. A dose response with each of these 3 reagents was observed. Zwittergen 3-14 showed almost complete solubilization using 0.1% or more detergent.

[00107] Antigen capture EIA was also used to determine the efficacy of Zwittergen extraction (FIGURE 13). An IgG preparation of an-rCARDS antisera was bound to plastic microtiter plate wells, γ-irradiated M. pneumoniae cells were adjusted to 1% Zwittergen followed by micro centrifugation. The supernatant was subjected to a five fold serial dilution series in a buffer containing 1% carrier protein (BSA), 1% Zwittergen 3-14 in PBS on the microtiter plate. The pellet after extraction was dispersed in the same buffer using the starting volume and was also serially diluted. Comparison of the curves indicates that the supernatant contained at least 25 fold more CARDS than the pellet. Additional plots reflect rCARDS spiked into various buffers serving as controls for the buffer formulations.

[00108] Example 6: Detection of M. pneumoniae in spiked specimens

[00109] Several throat and oral swabs were taken from one individual and placed into a buffer consisting of 1% Zwittergen 3-14 in PBS or in PBS alone, γ-irradiated M. pneumoniae were spiked into aliquots of these buffers and the spiked specimens were subjected to serial dilution. Negative control series were set up by spiking γ-irradiated M. salivarium, a commensal Mycoplasma found in human oral and respiratory specimens. Antigen capture EIA was performed using affinity purified anti-peptide 2 antibodies for capture and anti-rCARDS IgG-HRP conjugate antibodies for detection (FIGURE 14). A positive control employed rCARDS spiked into swab-conditioned 1% Zwittergen 3-14, PBS. Higher signal from M. pneumoniae is produced in the presence of detergent compared to swab conditioned PBS, confirming the efficacy of Zwittergen extraction (FIGURE 14). The lack of signal for M. salivarium indicates assay specificity.

[00110] Example 7: Detection of CARDS in Respiratory Specimens

[00111] Twenty-seven patient respiratory specimens were examined. These included transport media after elution of pharyngeal swabs, nasal aspirates, sputa, and broncho alveolar lavage (BAL) specimens. Nine specimens were purported to have been taken from patients with M. pneumoniae infection. Four specimens were from patients stated to have Chlamydia pneumoniae infection; no pathogen was stated for the remaining specimens. These specimens were adjusted to 0.1% Zwittergen 3-14 and were subjected to antigen capture EIA for the presence of CARDS. One of the 9 M. pneumoniae specimens and 18 non-M pneumoniae specimens each gave an EIA signal above background (data not shown).

[00112] Overall, these data suggest Zwittergen extraction of CARDS and antigen capture detection is a means of specifically detecting the presence of M. pneumoniae in a specimen.

(6) The scope of Claims

[Claim 1]

An isolated substance specifically binding to an epitope derived from M. Pneumoniae protein

[Claim 2]

The isolated substance of Claim 1, wherein the substance is an antibody

[Claim 3]

The isolated substance of Claim 1, wherein the substance is an antibody fragment

[Claim 4]

The isolated substance of any one of Claim 1, 2, or 3, wherein M. Pneumoniae protein is CARDS

[Claim 5]

The isolated substance of any one of Claim 1, 2, or 3, wherein M. Pneumoniae protein is P1

[Claim 6]

The isolated substance of Claim 4, wherein the epitope comprises any one of SEQ ID NOs: 2, 3, 4, 5, and 6

[Claim 7]

The isolated substance of Claim 5, wherein the epitope comprises SEQ ID NO: 8, 9, or 10

[Claim 8]

A method for detecting the presence of M. pneumoniae protein or bacterium in a sample, comprising contacting at least one isolated agent of any one of Claims 1 through 7 with a sample, and detecting a complex between the at least one isolated agent of any one of Claims 1 through 7 and the M. pneumoniae protein or bacterium

[Claim 9]

The method of Claim 8, further comprising the extraction of M. Pneumoniae protein from a sample

[Claim 10]

A device comprising a porous examination strip including at least one fraction comprising the substance of any one of Claims 1 to 7

[Claim 11]

The device of Claim 10, wherein one zone comprises a mobilizable agent of any one of Claims 1 through 7 and another zone comprises an immobilized agent of any one of Claims 1 through 7

[Claim 12]

The device of Claim 10, wherein the device is a lateral flow device

[Claim 13]

The device of Claim 10, wherein the device is an opposable-element, detachable-element, or multiple component chromatographic test device

3 Grounds 1 for rescission (Errors in the finding of cited invention as well as the identical features and the different features)

(1) In summary, Plaintiff's argument lies in that the patent invention is an invention that has first realized the specific detection of Mycoplasma pneumoniae from a clinical sample through immunochromatography by focusing on a monoclonal antibody specific to P1 protein, Cited reference 1 is a patent publication of an invention that focused on a protein (CARDS) totally different from P1 protein and its polyclonal

antibody, and furthermore, Cited reference 1 fails to detect Mycoplasma pneumoniae from a clinical sample even in a case of using a polyclonal antibody specific to CARDS, and first of all, when it comes to an antibody specific to P1 protein, it fails to conduct even a detection experiment using a purified rP1 protein as well as clinical sample; however, the revocation decision found the cited invention 1 as an invention capable of detecting Mycoplasma pneumoniae from a patient's sample (clinical sample) by using a monoclonal antibody specific to P1 protein, while overlooking any of the difference between P1 protein and CARDS protein, the difference between a clinical sample and a non-clinical sample, and further the difference between a monoclonal antibody and a polyclonal antibody.

(2) Therefore, at first, a consideration is given as to whether Cited invention 1 as the revocation decision found might be recognized from Cited reference 1.

The case in which the court determines that "an invention described in a publication" of Article 29, paragraph (1), item (iii) of the Patent Act should constitute a basis for the determination of whether a person skilled in the art could have easily conceived of the present invention (the patent invention) on the basis of the state of the art as of the filing, and thus should be a specific technical idea that could be extracted from the description of the publication. Further, the patent invention is an invention of a product, and thus a comparison is made with an invention of a product described in a publication in considering the inventive step. Here, it is necessary for a person skilled in the art to be able to make a product on the basis of the description of the publication and the common general knowledge as of the filing of the Patent (hereinafter referred to as "as of the filing") in order to find that an invention of a product is described in the publication.

A consideration is given to this case from such viewpoint, it cannot be said that the device of the cited invention 1 can be produced by a person skilled in the art even in view of the description of Cited reference 1 and the common general knowledge as of the filing. The reason is set forth as below.

A The revocation decision found the cited invention 1 as an invention of a lateral flow device to detect Mycoplasma pneumoniae in a patient's sample by use of monoclonal antibody against P1 protein. The lateral flow device is a detection device based on immunochromatography. In the antigen detection by immunochromatography, the formation of a sandwich complex between an antibody and an antigen is necessary (Exhibit Ko 8 to Ko 10, the entire import of the oral argument). Further, in a case of monoclonal antibody, it is inconvenient if two antibodies sandwiching an antigen are the same. Thus it is necessary to use two

different antibodies (in this regard, there is no dispute between the parties).

On the other hand, there is no common general knowledge as of the filing that two different monoclonal antibodies would form a sandwich complex between an antibody and an antigen, nor can it be directly inferred that the formation of a sandwich complex may always detect Mycoplasma pneumoniae in a patient's sample.

For example, Cited reference 2 shows on page 199 and Figure 1 two graphs showing a result of a test in which two polyclonal antibodies with different specificities as a capture antibody were used to implement a capture assay of Mycoplasma pneumoniae antigen by changing peroxidase-labeled monoclonal antibody (detection antibody). If a capture antibody is anti-MP-IgG (right), a tested peroxidase labeled antibody showed an absorbance at 450 nm beyond 2 for 100 ng of labeled antibody, and an absorbance at 450 nm beyond 3 for 1 μg of labeled antibody. In contrast, if a capture antibody is anti-P1-IgG (left), although a labeled antibody of P1.25 or M74 showed an absorbance at 450 nm beyond 3 for 1 μg, a labeled antibody of M57 only showed an absorbance of less than 1 for 1 μg. As described above, even in a case of using the same capture antibody, the detection sensitivity differs depending on a detection antibody. Further, it can be seen that the detection based on the formation of a sandwich complex differs in detection sensitivity depending on a combination of antibodies. Thus it is recognized that it is necessary to use a combination of proper antibodies in a case of implementing the detection on the basis of the formation of a sandwich complex by use of a monoclonal antibody.

The lateral flow device of the cited invention 1 as the revocation decision found is also a detection device of antigen based on the formation of a sandwich complex. It is thus recognized that it is necessary to use an appropriate combination of monoclonal antibodies as a first monoclonal antibody and a second monoclonal antibody to manufacture a lateral flow device for the detection of Mycoplasma pneumoniae in a patient's sample by using monoclonal antibody against P1 protein.

Accordingly, when a consideration is given to the description of cited reference 1 with regard to a combination of a first monoclonal antibody and a second monoclonal antibody, cited reference 1 is silent about a combination of a specific monoclonal antibody with regard to two antibodies for the use in a lateral flow device. Further, there is no evidence showing that a specific combination of monoclonal antibodies capable of forming a sandwich complex such as a lateral flow device was well-known as of the filing. (In an experiment shown in a graph on a left side of Figure 1 on page 199 of Cited reference 2, P1.25 and M74 are supposed to form sandwich complexes of a capture antibody and an antigen since an antigen is detectable given

that anti-P1-IgG or anti-Mp-IgG is a capture antibody; however, it cannot be directly deduced from the description of cited reference 2 that these antibodies are well-known. First of all, the capture antibodies are both polyclonal antibodies, and thus it cannot be said that a combination of two different monoclonal antibodies is clarified. There is no other evidence clarifying a specific combination of monoclonal antibodies capable of forming a sandwich complex.).

Subsequently, a sole example of a specific immunochromatography (ICT) device described in cited reference 1 of Example 4 used anti-rCARDS antibody, and did not use an antibody against P1 protein. Further, a specific description of antibody against P1 protein in cited reference 1 is only Example 3. The detection of antigen in Example 3 depends on a method using a commercially-available secondary antibody of antirabbit antibody or antimouse antibody; the method is different from the formation of a sandwich complex. Therefore, a monoclonal antibody capable of forming a sandwich complex could not be read from the description of these examples.

Furthermore, cited reference 1 describes a monoclonal antibody against monoclonal anti-genuine P1 protein antibody H136E7 ([0012]) and rP1 of mice ([0096]); it is only H136E7 that is described as a specific monoclonal against P1 protein. Regarding a monoclonal antibody against rP1, there is no cell line that produces the monoclonal antibody, no information on amino acid sequence, etc. of a monoclonal antibody and no information that gives a clue to the formation or non-formation of a sandwich complex with H136E7. In order to produce a lateral flow device on the basis of such description of cited reference 1, it is necessary to use H136E7 as one of monoclonal antibodies, and use another monoclonal antibody capable of forming a sandwich complex with H136E7. Cited reference 1 fails to give a clue to the structure of such monoclonal antibody. It is thus required to obtain monoclonal antibodies in some way, investigate whether these monoclonal antibodies may form a sandwich complex with H136E7, and discover a monoclonal antibody that can produce a lateral flow device for the detection of Mycoplasma pneumoniae in a patient's sample in combination with H136E7 by trial and error.

Taking the above into account, even if the technique to obtain various monoclonal antibodies was a matter of well-known art, it cannot be said that the lateral flow device of cited invention 1 as the revocation decision finds may be produced directly on the basis of the description of cited reference 1 and the common general knowledge as of the filing.

Therefore, it cannot be said that cited reference 1 describes (or substantially describes) the cited invention.

B A consideration is given to the detection from a patient's sample (clinical sample).

Cited reference 1 describes the detection of Mycoplasma pneumoniae in a patient's sample from a patient's sample in Example 7. This method is based on antigen-capture EIA with CARDS as an antigen for detection. Thus this is different in antigen and detection method from the device of the cited invention 1 that detects P1 protein on the basis of the formation of a sandwich complex. Furthermore, it is uncertain as to whether the infection might be detected from a sample, as set forth below.

Specifically, Cited reference 1 discloses in Example 7 that one of the 9 M. pneumoniae specimens and 18 non-M pneumoniae specimens each gave an EIA signal above background in a test with samples from patients. Here, Cited reference 1 only describes antigen-capture EIA, and fails to describe a specific detection system; however, if it were a common detection system based on the formation of a sandwich complex, a signal increases due to the presence of antigen, and thus a test result of Example 7 does not correctly associate the infection or non-infection with an increase in the signal.

In this regard, Defendant argues that the competition method is a detection method in which a signal gets decreased with increasing antigen in a sample, and it is thus expected that a signal gets increased due to the absence of antigen for 18 samples of non-infection of M. pneumoniae, whereas a signal gets decreased due to large amount of antigen for 9 samples of M. pneumoniae infection, Example 7 coincides with this expectation, and thus Example 7 should be construed as demonstrating the detection of infection/non-infection.

However, supposing that the test of Example 7 be in compliance with a competition method, the competition method needs to use a labeled antigen, differing from the detection method using a labeled antibody such as a sandwich method using a labeled antibody, whereas cited reference 1 fails to describe the manufacture of the labeled antigen, or access to the labeled antigen. Further, first of all, the cited reference 1 fails to describe the detection system of Example 7. Therefore, it cannot be asserted from the consistency with a test result that the test is based on a competition method.

In view of the above, it cannot be said that the description of Example 7 of cited reference 1 shows that the detection of Mycoplasma pneumoniae from a patient's sample (clinical sample) was possible.

Also from this viewpoint, it cannot be said that cited reference 1 describes (or substantially describes) the cited invention.

(3) Summary

As aforementioned, in determining the inventive step, the revocation decision overlooked the different features with respect to the use of monoclonal antibodies as a first antibody and a second antibody and with respect to the detection of Mycoplasma pneumoniae in a patient's sample as a consequence of making an error in the finding of the cited invention, and it reached a conclusion of lack of inventive step without making a determination of whether these different features were easily conceivable, and revoked the patent (for this reason). Therefore, the errors in the finding of the cited invention and the overlooking of the different features affect the conclusion of the revocation decision.

Therefore, the grounds 1 for rescission as Plaintiff argues have a point as far as the above. Without considering the remaining grounds for rescission, the revocation decision should be rescinded.

4 Conclusion

Therefore, the revocation decision shall be rescinded, and the court sentences as in the main text.

Intellectual Property High Court, Third Division

Presiding Judge:	TSURUOKA Toshihiko
Judge:	TERADA Toshihiko
Judge:	MAGIRA Hiromitsu