

DESCRIPTION

METHOD FOR TESTING PRESENCE OR ABSENCE OF MYCOPLASMA

Technical Field

5 [0001]

The present invention relates to a method for testing presence or absence of a mycoplasma.

Background Art

[0002]

10 Mycoplasmas are well known as typical contaminating microorganisms of cultured cells. The mycoplasmas generally indicate microorganisms belonging to the class Mollicutes, are minimum in microorganisms capable of self-renewal without being parasitic in cells, and show
15 polymorphism and filterability. In addition, the mycoplasmas have no cell wall and thus are resistant to β -lactam antibiotic such as penicillin. The mycoplasmas are difficult to find out with typical microscope observation and contamination thereof is difficult to determine by
20 visual inspection, and thus the mycoplasmas are cultured or subcultured without noticing contamination in many cases. As influences of mycoplasma contamination, there are mentioned metabolic abnormality of cultured cells which are infected, cytokine induction, chromosomal variation, and
25 the like. In human bodies, it is known that a cytosolic

membrane surface protein of a mycoplasma acts as an antigen to affect the immunity system such as inducing inflammatory cytokines. Further, there is a concern that some of mycoplasmas cause diseases such as pneumonia, urethritis, and arthritis. Therefore, it is necessary to perform a mycoplasma detection test in the fields of regenerative medicine using live cells or tissues, cell therapy not intended to regenerate organs or tissues (for example, cancer cell immunotherapy), cell or tissue processing products used for processing such as cultivation, activation, and differentiation induction, cell processed products, vaccines, antibody drugs, and the like, in which production is performed using biomedical materials derived from animals.

[0003]

In The Japanese Pharmacopoeia 17th Edition (JP17), as the mycoplasma detection test, A. Cultural method, B: DNA staining method, and C. Nucleic acid amplification test (NAT) method are described. The cultural method needs a long culture period of 28 days in total, that is, cultivation in a liquid culture medium for 14 days and subsequently cultivation in an agar medium for 14 days. The DNA staining method needs about seven days as a period of time necessary for a test, but has low sensitivity and needs 1 mL or more of a specimen. Further, in the nucleic

acid amplification test method, a period of time necessary for a test is about several days, but typically needs about 1 to 10 mL of a specimen. As another method, a method of replacing cultivation in an agar medium for 14 days in the cultural method with detection by polymerase chain reaction (PCR) has been reported (Non Patent Literature 1).

However, even in this method, cultivation for about 14 days is necessary and about 1 mL of a specimen is necessary.

10 Citation List

Non Patent Literature

[0004]

Non Patent Literature 1: "Detection of live mycoplasmas by a combined method of preculture and PCR" Annual Report of the National Veterinary Assay Laboratory, vol. 44, 13 to 20 (2007)

Summary of Invention

Technical Problem

20 [0005]

An object of the present invention is to provide a method for testing presence or absence of a mycoplasma.

Solution to Problem

25 [0006]

In production of regenerative medicine products, there is a case where the amount of final products that can be produced may be limited, for example, the case of using autologous cells of patients, or the like. Further, the lifetime of the products is significantly shorter than general pharmaceutical products. Therefore, in the regenerative medicine products, unlike general pharmaceutical products, it is desirable that the amount of a specimen necessary for a quality test is small and the period of time necessary for the test is also short.

Despite this situation, in the mycoplasma detection test, as described above, the period of time necessary for duration of the test in the cultural method is 28 days, that is, a longer period of time than the lifetime of products is required, a large amount, that is, 1 mL or more, of a specimen is required in the DNA staining method, the nucleic acid amplification test method, and an improved cultural method, and thus all of these methods are not preferable methods in the regenerative medicine field.

[0007]

The present inventors have found during researches on a method for testing presence or absence of a mycoplasma that by incorporating a step of culturing a mycoplasma before the step of amplifying a nucleic acid in the nucleic acid amplification test method, existence of the mycoplasma can

be detected from a small amount of a specimen, thereby completing a new testing method of a mycoplasma which can be advantageously used in regenerative medicine products. As a result of their further researches on such a method, 5 the present inventors have encountered a new problem in that mixing of mycoplasma nucleic acid fragments from a biological material or the like contained in products or testing materials which is not problematic in the cultural method and the DNA staining method may cause false 10 positives. With respect to such problems, the present inventors have incorporated a step of removing impurities before a step of amplifying a nucleic acid and thus obtained new finding that false positives can be reduced. Then, researches are further continued based on such 15 finding, and as a result, the present invention is completed.

[0008]

That is, the present invention relates to the following.

20 [1] A method for testing presence or absence of a mycoplasma in a specimen, the method including:

(a) a step of culturing a specimen; and

(b) a step of extracting a nucleic acid from the cultured specimen and performing amplification of the

25 nucleic acid using a primer capable of amplifying a nucleic

acid sequence derived from a mycoplasma,

in which the specimen is a biotechnology-related product or a part thereof.

[2] The method described in [1], in which the specimen
5 is a regenerative medicine-related product or a part thereof.

[3] The method described in [1] or [2], in which the step of performing amplification of the nucleic acid is performed by a polymerase chain reaction (PCR) method.

10 [4] The method described in any one of [1] to [3], further including (c) a step of performing electrophoresis of an amplified product obtained from the step (b).

[5] The method described in any one of [1] to [4], in which the method includes performing anaerobic cultivation
15 in the step (a).

[0009]

[6] The method described in any one of [1] to [4], in which in the step (a), two or more specimens are each cultured in an aerobic or anaerobic condition.

20 [7] The method described in any one of [1] to [6], in which an amount of the specimen cultured in the step (a) is 10 μ L to 10 mL.

[8] The method described in any one of [1] to [7], in which in the step (a), cultivation is performed for 3 to 14
25 days.

[9] The method described in any one of [1] to [8], further including removing impurities before the nucleic acid is extracted in the step (b).

[10] The method described in any one of [1] to [9], in
5 which removing of impurities is performed by collecting the specimen with a filter.

[0010]

[11] The method described in any one of [1] to [10], in which a molecular weight cutoff of a filter is 1000 kDa or
10 less or a pore size of a filter is 0.22 μm or less.

[12] The method described in any one of [1] to [11], in which the regenerative medicine-related product includes at least one selected from a cell subjected to processing, a cell to be provided to processing, a reagent to be used in
15 processing or testing, a culture medium for culturing a cell, a cell washing solution for washing a cell, and a preservation liquid for preserving a regenerative medicine product.

[13] A method of managing quality of a biotechnology-
20 related product, the method including:

a step of testing presence or absence of a mycoplasma in a specimen by the method described in any one of [1] to [12].

[14] A kit for use in the method described in any one
25 of [1] to [12], the kit including: a culture medium for

culturing a mycoplasma; and a primer capable of amplifying a nucleic acid sequence derived from a mycoplasma.

[15] The kit described in [14], further including a filter for removing impurities.

5 [0011]

[I] A method for testing presence or absence of a mycoplasma in a specimen, the method including:

(i) a step of removing impurities from a composition containing a specimen; and

10 (ii) a step of extracting a nucleic acid from the composition containing a specimen from which impurities have been removed and performing amplification of the nucleic acid using a primer capable of amplifying a nucleic acid sequence derived from a mycoplasma.

15 [II] The method described in [I], in which the step of performing amplification of the nucleic acid is performed by a polymerase chain reaction (PCR) method.

[III] The method described in [I] or [II], further including (iii) a step of performing electrophoresis of an
20 amplified product obtained from the step (ii).

[IV] The method described in any one of [I] to [III], in which in the step (i), the step of removing impurities is performed by collecting the specimen with a filter.

[V] The method described in any one of [I] to [IV], in
25 which a molecular weight cutoff of a filter is 1000 kDa or

less or a pore size of a filter is 0.22 μm or less.

[0012]

[VI] The method described in any one of [I] to [V], in which the method includes culturing the specimen before the
5 step (ii).

[VII] The method described in any one of [I] to [VI], in which the method includes culturing the specimen before the step (i).

[VIII] The method described in any one of [I] to [VII],
10 in which the specimen is a biotechnology-related product or a part thereof.

[IX] The method described in any one of [I] to [VIII], in which the specimen is a regenerative medicine-related product or a part thereof.

15 [X] The method described in any one of [I] to [IX], in which the regenerative medicine-related product includes at least one selected from a cell subjected to processing, a cell to be provided to processing, a reagent to be used in processing or testing, a culture medium for culturing a
20 cell, a cell washing solution for washing a cell, and a preservation liquid for preserving a regenerative medicine product.

[XI] A method of managing quality of a biotechnology-related product, the method including:

25 a step of testing presence or absence of a mycoplasma

in a specimen by the method described in any one of [I] to [X].

[XII] A kit for use in the method described in any one of [I] to [X], the kit including: a primer capable of
5 amplifying a nucleic acid sequence derived from a mycoplasma; and a filter for removing impurities.

Advantageous Effects of Invention

[0013]

10 According to the present invention, it is possible to detect a mycoplasma with sensitivity of 10 cfu/mL or less in a short time even from a small amount of a specimen. Since a period of time required for a test is sufficient to be short and an amount of a specimen to be provided to the
15 test is sufficient to be small, particularly in the biotechnology field including regenerative medicine, this method is extremely useful. Further, by incorporating the step of removing impurities before the step of amplifying a nucleic acid, the test with reduced false positives and
20 high accuracy can be performed.

Brief Description of Drawings

[0014]

Fig. 1 is an electrophoretic profile of a PCR product.
25 In (A), M shows a 100 bp ladder, Lane 1 shows the result

when PCR is performed without addition of a mycoplasma, and Lane 2 shows a positive control (the result when PCR is performed with addition of a mycoplasma (*Acholeplasma laidlawii*)). In (B), M shows a 100 bp ladder, Lanes 1 to 3 show the result when PCR is performed without addition of a mycoplasma after collection with a filter, and Lanes 4 to 6 show a positive control (the result when PCR is performed with addition of a mycoplasma (*Acholeplasma laidlawii*) after collection with a filter).

Fig. 2 is a graph showing proliferation of a mycoplasma in a cellular specimen. (A) shows a result of aerobic cultivation and (B) shows a result of anaerobic cultivation. The vertical axis shows a concentration (cfu/mL, logarithmic expression) of a mycoplasma and the horizontal axis shows cultivation days (day).

Fig. 3 is a graph showing proliferation of a mycoplasma in a culture medium specimen. (A) shows a result of aerobic cultivation and (B) shows a result of anaerobic cultivation. The vertical axis shows a concentration (cfu/mL, logarithmic expression) of a mycoplasma and the horizontal axis shows cultivation days (day).

Fig. 4 is a graph showing proliferation of a mycoplasma in a washing solution specimen. (A) shows a result of aerobic cultivation and (B) shows a result of anaerobic cultivation. The vertical axis shows a concentration

(cfu/mL, logarithmic expression) of a mycoplasma and the horizontal axis shows cultivation days (day).

[0015]

Fig. 5 shows proliferation and a detection limit of a mycoplasma in a cellular specimen. The vertical axis shows a concentration (cfu/mL, logarithmic expression) of a mycoplasma. In the drawing, black represents a concentration of a mycoplasma after cultivation for 7 days and gray represents detection sensitivity.

Fig. 6 shows proliferation and a detection limit of a mycoplasma in a culture medium specimen. The vertical axis shows a concentration (cfu/mL, logarithmic expression) of a mycoplasma. In the drawing, black represents a concentration of a mycoplasma after cultivation for 7 days and gray represents detection sensitivity.

Fig. 7 shows proliferation and a detection limit of a mycoplasma in a washing solution specimen. The vertical axis shows a concentration (cfu/mL, logarithmic expression) of a mycoplasma. In the drawing, black represents a concentration of a mycoplasma after cultivation for 7 days and gray represents detection sensitivity.

Fig. 8 is an electrophoretic profile of a PCR product. M shows a 100 bp ladder, Lane 1 shows a negative control (the result when PCR is performed with respect to water), Lanes 2 to 4 show a negative control (the result when PCR

is performed by aerobic cultivation using PBS instead of a specimen), Lanes 5 to 7 show the result when PCR is performed by aerobic cultivation of a specimen, Lanes 8 to 10 show a positive control (the result when PCR is performed by aerobic cultivation with addition of a mycoplasma diluent (*Mycoplasma orale*) to a specimen), Lanes 11 to 13 show a negative control (the result when PCR is performed by anaerobic cultivation using PBS instead of a specimen), Lanes 14 to 16 show the result when PCR is performed by anaerobic cultivation of a specimen, and Lanes 17 to 19 show a positive control (the result when PCR is performed by anaerobic cultivation with addition of a mycoplasma diluent (*Mycoplasma salivarium*) to a specimen).

15 Description of Embodiments

[0016]

Hereinafter, the present invention will be described in detail.

Unless defined otherwise in this specification, all the technical terms and scientific terms used in this specification have the same meanings as usually understood by those skilled in the art. All the patents, applications, and other publications or information referred to in this specification are incorporated in this specification by reference in their entirety. Further, if

any contradiction arises between publications referred to in this specification and descriptions in this specification, the descriptions in this specification supersede.

5 [0017]

The present invention relates to a method for testing presence or absence of a mycoplasma in a specimen, the method including: a step of extracting a nucleic acid from a specimen and performing amplification of the nucleic acid
10 using a primer capable of amplifying a nucleic acid sequence derived from a mycoplasma.

[0018]

In the present invention, the "specimen" is not particularly limited as long as it is an arbitrary target
15 in which mixing of a mycoplasma is desired to be tested, a target in which there is a concern of mycoplasma contamination, a part thereof, or the like. Non-limiting examples thereof include biomedical materials of arbitrary biological origin (for example, tissues, cells, biologic
20 fluids, and the like) and products produced using these materials as raw materials (for example, some of organs, fusion cells, pharmaceutical products of biological origin, and the like). Specific examples thereof include animal-derived biomedical materials and products produced using
25 these materials as raw materials, and those which are

provided for tests or production performed using animal-derived biomedical materials. Examples of the animal-derived biomedical materials and products produced using these materials as raw materials include, although not

5 limited thereto, cells, tissues, blood (whole blood or some of whole blood, for example, including serum, blood plasma, and the like), placenta, urine, and antibodies of animals, biological preparations (for example, proteins, polypeptide, derivatives thereof, and pharmaceutical
10 products, antibody pharmaceutical products, vaccines, antitoxins, blood preparations, and the like which use proteins, polypeptide, or derivatives thereof as constituents), and regenerative medicine products.

Examples of those which are provided for tests or
15 production performed using animal-derived biomedical materials include, although not limited thereto, tools which are provided for tests or production performed using animal-derived biomedical materials (for example, cell culture vessels and the like), reagents, serum, antibodies,
20 culture media, and washing solutions.

Further, the "specimen" of the present invention may be those which are produced as a biotechnology-related product or a regenerative medicine-related product.

[0019]

25 In the present invention, the "biotechnology-related

product" includes a biological preparation, a regenerative medicine product, or a product and the like which are used in production thereof.

In the present invention, the "biological preparation" includes, for example, proteins, polypeptide, derivatives thereof, and pharmaceutical products, antibody pharmaceutical products, vaccines, antitoxins, blood preparations, and the like which use proteins, polypeptide, or derivatives thereof as constituents.

In the present invention, a product used in production of a biological preparation includes a cell producing a recombination protein or a polypeptide, blood (whole blood or some of whole blood, for example, including serum, blood plasma, and the like), placenta, urine, a culture medium for culturing a cell, a cell washing solution for washing a cell, a preservation liquid for preserving a biological preparation, a reagent to be used in processing or testing (for example, a buffer solution, a detection reagent, serum, a growth factor, an yeast extract, or the like), and the like.

Examples of the cell producing a recombination protein or a polypeptide include E. coli, yeast, insect cells, plant cells, hybridoma, animal cells (for example, CHO cells, Sp2/0 cells, NS0 cells, and the like), and human cells. In the present invention, the product used in

production of a biological preparation can include a vaccine, but the vaccine can also be excluded from the product used in production of a biological preparation.
[0020]

5 In the present invention, the "regenerative medicine-related product" includes a regenerative medicine product or a product and the like which are used in production of the regenerative medicine product.

10 In the present invention, the "regenerative medicine product" is (1) a product which is obtained by subjecting cells of humans or animals to processing such as cultivation, and which (a) reconstructs/restores/forms a structure/function of a body or (b) is used for medical treatment/prevention of diseases, or is (2) a product which
15 is introduced into cells of a human and then used for the purpose of gene therapy. The "regenerative medicine product" includes a cell used in medical treatment as a body, and for example, includes a cell subjected to processing and a cell to be provided to processing.
20 Specifically, non-limiting examples thereof include human (allogeneic) bone marrow-derived mesenchymal stem cells used in medical treatment of acute graft-versus-host disease (acute GVHD) after hematopoietic stem cell transplantation, human (autologous) skeletal muscle-derived
25 cell sheets to be transplanted in medical treatment of

serious heart failures, and cells to be used in cancer immunotherapy (such as dendritic cells, NK cells, and T lymphocytes).

In the present invention, a product used in production
5 of the regenerative medicine product includes a reagent to be used in processing or testing (for example, a buffer solution, a detection reagent, serum, a growth factor, an yeast extract, or the like), a culture medium for culturing a cell, a cell washing solution for washing a cell, a
10 preservation liquid for preserving a regenerative medicine product, and the like.

[0021]

In the present invention, the cell subjected to processing and the cell to be provided to processing are
15 animal cells, preferably mammal cells, and more preferably human cells.

Non-limiting examples of the cell subjected to processing and the cell to be provided to processing include somatic cells (for example, myocardial cells, fibroblasts, epithelial cells, endothelial cells, hepatic
20 cells, pancreatic cells, renal cells, adrenal cells, periodontal ligament cells, gingival cells, periosteal cells, skin cells, synovial cells, cartilage cells, dendritic cells, lymphocytes (natural killer (NK) cells, T
25 lymphocytes, and B lymphocytes), and the like), and stem

cells (for example, tissue stem cells such as myoblasts and cardiac stem cells, embryonic stem cells (ES) cells), pluripotent stem cells such as induced pluripotent stem (iPS) cells, mesenchymal stem cells, and the like). The somatic cells may be stem cells, in particular, cells differentiated from iPS cells, and specific examples thereof include iPS-derived myocardial cells, iPS-derived dendritic cells, and iPS-derived lymphocytes (NK cells, T lymphocytes, and B lymphocytes). These cells may be modified by gene introduction, and specific examples of such cells include a chimeric antigen receptor-T cell (CAR-T) and a T cell receptor-T cell (TCR-T).

The cell subjected to processing refers to a cell obtainable after subjected to some kind of processing, and the cell to be provided to processing refers to a cell to be prepared before subjected to some kind of processing. The processing is not limited as long as it is any operations applied to cells, and for example, may be processing for treatment of diseases. Specific examples of such cells include cells cultured in a liquid, cells cultured in the environment, for example, in a gel, cell cultures cultured and then three-dimensionally constructed via cellular adhesion, sheet-shaped cell cultures, cell suspensions in which a cell density is adjusted, cells cultured and grown, cells cultured and provided to

freezing, cells melted from a freeze state, cells in which the number of cells or density thereof is adjusted, and cells to be set for administration in an administration device.

5 [0022]

Diseases to be treated by such cells are not limited, and examples thereof include cardiac diseases, lung diseases, liver diseases, pancreatic disorders, kidney diseases, large intestine diseases, small intestine
10 diseases, spinal cord diseases, central nervous system diseases, bone diseases, eye diseases, and skin diseases. Cancers (malignant neoplasm) such as blood cancer and solid cancer are included in diseases. In a case where cells to be used are myoblasts or (iPS-derived) myocardial cells,
15 examples of diseases include cardiac infarction (including chronic cardiac failure accompanied by cardiac infarction), dilated cardiomyopathy, ischemic cardiomyopathy, and cardiac diseases (for example, cardiac failure, particularly chronic cardiac failure) accompanied by
20 systolic functional disorder (for example, left systolic functional disorder).

[0023]

In an embodiment of the present invention, the regenerative medicine-related product includes at least one
25 selected from a cell subjected to processing, a cell to be

provided to processing, a reagent to be used in processing or testing, a culture medium for culturing a cell, a cell washing solution for washing a cell, and a preservation liquid for preserving a regenerative medicine product.

5 Specific examples thereof include a sheet-shaped cell culture, a cell for producing a sheet-shaped cell culture (for example, a cryopreserved cell or the like), a sheet preparation culture medium, and a cell washing solution.

In an embodiment of the present invention, the
10 regenerative medicine-related product includes at least one selected from a cell subjected to processing and a cell to be provided to processing.

In an embodiment of the present invention, the regenerative medicine-related product includes at least one
15 selected from myoblasts and iPS-derived myocardial cells.

In an embodiment of the present invention, the regenerative medicine-related product includes at least one selected from mesenchymal stem cells and fibroblasts.

In an embodiment of the present invention, the
20 regenerative medicine-related product includes at least one selected from dendritic cells, NK cells, T lymphocytes, B lymphocytes, CAR-T, and TCR-T.

[0024]

In the present invention, the sheet-shaped cell culture
25 refers to cells linked together into the shape of a sheet.

Cells may be linked together directly (including cellular adhesion linked via cellular elements, such as adhesion molecules) and/or linked together via an intervening substance. The intervening substance is not particularly limited as long as it is a substance having at least an ability to physically (mechanically) link cells together, and examples thereof include extracellular matrices. The intervening substance may preferably be a substance derived from cells, in particular, a substance derived from cells constituting the cell culture. Although the cells are linked at least physically (mechanically), the cells may be further linked functionally, for example, chemically or electrically. The sheet-shaped cell culture may be a culture configured with a single cell layer (single layer), or a culture configured with two or more cell layers (stacked (multilayer) architecture, for example, two layers, three layers, four layers, five layers, six layers, and the like).

[0025]

In the present invention, the sheet-shaped cell culture is used in medical treatment of humans or animals, and cells constituting the sheet-shaped cell culture are animal cells, preferably mammal cells, and more preferably human cells.

In the present invention, the cells constituting the

sheet-shaped cell culture are not particularly limited as long as they can form the sheet-shaped cell culture, and for example, include adhesion cells (adherent cells).

Examples of the adhesion cells include adherent somatic

5 cells (for example, myocardial cells, fibroblasts, epithelial cells, endothelial cells, hepatic cells, pancreatic cells, renal cells, adrenal cells, periodontal ligament cells, gingival cells, periosteal cells, skin cells, synovial cells, cartilage cells, and the like) and
10 stem cells (for example, tissue stem cells such as myoblasts and cardiac stem cells, embryonic stem cells, pluripotent stem cells such as induced pluripotent stem (iPS) cells, mesenchymal stem cells, and the like). The somatic cells may be stem cells, in particular, cells
15 differentiated from iPS cells.

Further, non-limiting examples of the cells constituting the sheet-shaped cell culture include myoblasts (for example, skeletal myoblasts and the like), mesenchymal stem cells (for example, bone marrow, adipose
20 tissue, peripheral blood, skin, hair root, muscular tissue, endometrium, placenta, cord blood-derived cells, and the like), myocardial cells, fibroblasts, cardiac stem cells, embryonic stem cells, iPS cells, synovial cells, cartilage cells, epithelium cells (for example, oral mucosaepithelium
25 cells, retinal pigment epithelial cells, nasal epithelial

cells, and the like), endothelial cells (for example, vascular endothelial cells and the like), hepatocytes (for example, hepatic parenchymal cells and the like), pancreatic cells (for example, islet cells and the like),
5 renal cells, adrenal gland cells, human periodontal ligament cells, gingiva cells, periosteum cells, and skin cells. The sheet-shaped cell culture is configured by these cells, and specific examples thereof include a skeletal myoblast sheet, an iPS-derived myocardial cell
10 sheet, a mesenchymal stem cell sheet, and a fibroblast sheet.

[0026]

In the present invention, the mycoplasma is not particularly limited as long as it is a bacterium
15 classified in the class Mollicutes. Specific examples thereof include, although not particularly limited, bacteria belonging to the genus *Mycoplasma* in the family Mycoplasmataceae, the genus *Acholeplasma* in the family *Acholeplasmataceae*, the genus *Spiroplasma* in the family
20 *Spiroplasmataceae*, and the like. More specific examples thereof include *Mycoplasma arginini*, *Mycoplasma salivarium*, *Mycoplasma pneumoniae*, *Mycoplasma fermentans*, *Mycoplasma orale*, *Mycoplasma hyorhinitis*, *Mycoplasma synoviae*, *Acholeplasma laidlawii*, and *Spiroplasma citri*. In this
25 specification, *Mycoplasma arginini* is also referred to as

Ma, *Mycoplasma salivarium* is also referred to as Ms, *Mycoplasma pneumoniae* is also referred to as Mp, *Mycoplasma fermentans* is also referred to as Mf, *Mycoplasma orale* is also referred to as Mo, *Mycoplasma hyorhinis* is also referred to as Mh, and *Acholeplasma laidlawii* is also referred to as Al.

[0027]

In the present invention, it is sufficient that the amount of a specimen is an amount necessary for amplifying a mycoplasma to an amount beyond the detection sensitivity of this method, and it is possible for those having ordinary knowledge in the relevant technical field to set an appropriate amount. Non-limiting examples thereof include 10 μ L, 15 μ L, 20 μ L, 25 μ L, 30 μ L, 35 μ L, 40 μ L, 45 μ L, 50 μ L, 55 μ L, 60 μ L, 65 μ L, 70 μ L, 75 μ L, 80 μ L, 85 μ L, 90 μ L, 95 μ L, 100 μ L, 110 μ L, 120 μ L, 130 μ L, 140 μ L, 150 μ L, 160 μ L, 170 μ L, 180 μ L, 190 μ L, 200 μ L, 250 μ L, 300 μ L, 350 μ L, 400 μ L, 450 μ L, 500 μ L, 550 μ L, 600 μ L, 650 μ L, 700 μ L, 750 μ L, 800 μ L, 850 μ L, 900 μ L, 950 μ L, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL, and 10 mL.

[0028]

In the present invention, the lower limit of the amount of a specimen is not particularly limited as long as it is an amount necessary for amplifying a mycoplasma to an amount beyond the detection sensitivity of this method.

Non-limiting examples thereof include 10 μ L or more, 15 μ L or more, 20 μ L or more, 25 μ L or more, 30 μ L or more, 35 μ L or more, 40 μ L or more, 45 μ L or more, 50 μ L or more, 55 μ L or more, 60 μ L or more, 65 μ L or more, 70 μ L or more, 75 μ L or more, 80 μ L or more, 85 μ L or more, 90 μ L or more, 95 μ L or more, and 100 μ L or more. Preferably, the amount of a specimen is 100 μ L.

[0029]

In the present invention, the upper limit of the amount of a specimen is not particularly limited, but in the case of using the method of the present invention in a regenerative medicine-related product, among amounts in which the detection sensitivity of this method can be achieved, the amount is preferably an amount as small as possible. Non-limiting examples thereof include 10 mL or less, 9 mL or less, 8 mL or less, 7 mL or less, 6 mL or less, 5 mL or less, 4 mL or less, 3 mL or less, 2 mL or less, 1 mL or less, 950 μ L or less, 900 μ L or less, 850 μ L or less, 800 μ L or less, 750 μ L or less, 700 μ L or less, 650 μ L or less, 600 μ L or less, 550 μ L or less, 500 μ L or less, 450 μ L or less, 400 μ L or less, 350 μ L or less, 300 μ L or less, 250 μ L or less, 200 μ L or less, 150 μ L or less, and 100 μ L or less.

[0030]

In the present invention, as for the range of the

amount of a specimen, arbitrary combinations of the upper limits and the lower limits exemplified above are mentioned. That is, examples thereof include 10 μ L to 10 mL, 10 μ L to 9 mL, 10 μ L to 8 mL, 10 μ L to 7 mL, 10 μ L to 6 mL, 10 μ L to 5 mL, 10 μ L to 4 mL, 10 μ L to 3 mL, 10 μ L to 2 mL, 10 μ L to 1 mL, 10 to 900 μ L, 10 to 800 μ L, 10 to 700 μ L, 10 to 600 μ L, 10 to 500 μ L, 10 to 400 μ L, 10 to 300 μ L, 10 to 200 μ L, 10 to 100 μ L, 100 μ L to 10 mL, 100 μ L to 9 mL, 100 μ L to 8 mL, 100 μ L to 7 mL, 100 μ L to 6 mL, 100 μ L to 5 mL, 100 μ L to 4 mL, 100 μ L to 3 mL, 100 μ L to 2 mL, 100 μ L to 1 mL, 100 to 900 μ L, 100 to 800 μ L, 100 to 700 μ L, 100 to 600 μ L, 100 to 500 μ L, 100 to 400 μ L, 100 to 300 μ L, and 100 to 200 μ L. The range thereof is particularly preferably 100 to 1000 μ L and further preferably 100 to 500 μ L.

[0031]

In the present invention, the nucleic acid is DNA or RNA, and is preferably DNA.

In the present invention, the method of extracting a nucleic acid from a specimen is not particularly limited as long as it can extract a nucleic acid of a mycoplasma, and it is possible for those skilled in the art to appropriately select an optimal method. For example, phenol/chloroform extraction, an extraction method using a carrier, and the like may be employed. Extraction of a

nucleic acid may be performed using a commercially available kit for nucleic acid extraction.

[0032]

In the present invention, the method of amplifying a
5 nucleic acid is not particularly limited as long as it can
amplify a nucleic acid sequence derived from a mycoplasma,
and it is possible for those skilled in the art to
appropriately select an optimal method.

In the present invention, examples of the method of
10 amplifying a nucleic acid include a polymerase chain
reaction (PCR) method and a loop-mediated isothermal
amplification (LAMP) method.

In an embodiment of the present invention, the step of
performing amplification of the nucleic acid is performed
15 by a polymerase chain reaction (PCR) method. In the PCR
method, a primer capable of amplifying a nucleic acid
sequence derived from a mycoplasma, polymerase, a buffer,
PCR conditions (temperature, time, the number of cycles,
and the like), and the like can be appropriately selected.

20 In the present invention, PCR may be, for example,
regular PCR, real-time PCR, Nested PCR, reverse
transcription PCR, or the like. Regular PCR is preferred.

[0033]

As described above, as for the primer in the present
25 invention, any primers may be used as long as they can

amplify a nucleic acid sequence derived from a mycoplasma. Non-limiting examples of the primer capable of amplifying a nucleic acid sequence derived from a mycoplasma include the following sequences:

5 Forward: 5' -GGCGAATGGGTGAGTAACACG- 3', and
 Reverse: 5' -CGGATAACGCTTGCGACCTATG- 3'.

[0034]

 An aspect of the present invention relates to a method for testing presence or absence of a mycoplasma in a
10 specimen, the method including:

 (a) a step of culturing a specimen; and
 (b) a step of extracting a nucleic acid from the
cultured specimen and performing amplification of the
nucleic acid using a primer capable of amplifying a nucleic
15 acid sequence derived from a mycoplasma,

 in which the specimen is a biotechnology-related
product or a part thereof.

[0035]

 In an embodiment of the present invention, the specimen
20 is a regenerative medicine-related product or a part
thereof.

 In an embodiment of the present invention, the
regenerative medicine-related product includes at least one
selected from a cell subjected to processing, a cell to be
25 provided to processing, a reagent to be used in processing

or testing, a culture medium for culturing a cell, a cell washing solution for washing a cell, and a preservation liquid for preserving a regenerative medicine product.

Specific examples thereof include a sheet-shaped cell

5 culture, a cell for producing a sheet-shaped cell culture (for example, a cryopreserved cell or the like), a sheet preparation culture medium, and a cell washing solution.

[0036]

In an embodiment of the present invention, this method
10 further includes (c) a step of performing electrophoresis of an amplified PCR product obtained from the step (b).

In the present invention, the electrophoresis of the amplified product is not particularly limited as long as it is a method with which the amplified nucleic acid can be
15 detected, and it is possible for those skilled in the art to appropriately select an optimal method. For example, as for a method for preparing a gel such as agarose gel, selection of a buffer, and a method of checking a band, it is possible for those skilled in the art to appropriately
20 select an optimal method. Examples of the method of checking a band include staining of a nucleic acid with ethidium bromide, SYBR (registered trademark) Green, or the like and a southern blotting method.

[0037]

25 In the present invention, conditions of the cultivation

are not particularly limited as long as a method can amplify a mycoplasma, and it is possible for those skilled in the art to appropriately select an optimal method. For example, types and composition of a culture medium, a
5 cultivation temperature, an atmosphere, a CO₂ concentration, and the like can also be appropriately selected.

In the present invention, cultivation may be performed by aerobic cultivation or anaerobic cultivation and can be
10 appropriately selected according to types of a mycoplasma to be tested. For example, as for five kinds of *Mycoplasma arginini*, *Mycoplasma pneumoniae*, *Mycoplasma fermentans*, *Mycoplasma orale*, and *Acholeplasma laidlawii*, these mycoplasmas can be amplified under any of an aerobic
15 condition and an anaerobic condition; however, since *Mycoplasma salivarium* can be amplified only under an anaerobic condition and *Mycoplasma hyorhinae* can be amplified only under an aerobic condition, six kinds other than *Mycoplasma salivarium* may be cultured under an aerobic
20 condition and *Mycoplasma salivarium* may be cultured under an anaerobic condition. In order to perform testing of all seven kinds, two or more specimens can be each cultured in an aerobic or anaerobic condition.

In an embodiment of the present invention, this method
25 includes performing anaerobic cultivation in the step (a).

In an embodiment of the present invention, in this method, two or more specimens are each cultured in an aerobic or anaerobic condition.

[0038]

5 In an embodiment of the present invention, an amount of a specimen to be cultured in the step (a) is 10 μ L to 10 mL.

In an embodiment of the present invention, an amount of a specimen to be cultured in the step (a) is 100 μ L to 1
10 mL.

In an embodiment of the present invention, an amount of a specimen to be cultured in the step (a) is 100 μ L to 500 μ L.

[0039]

15 In the present invention, the period of time for cultivation may be a period which is sufficient for amplifying a mycoplasma to an amount beyond the detection sensitivity obtained by this method, and can be arbitrarily set. Non-limiting examples of the period of time for
20 cultivation include 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, and 14 days. Non-limiting examples of the range of the period of time for cultivation include 1 day to 14 days, 1 day to 7 days, 3 days to 14 days, 3 days to 7 days,
25 and 6 days to 10 days. The range thereof is preferably 3

days to 14 days and more preferably 6 days to 10 days.

In an embodiment of the present invention, cultivation is performed for 6 days to 10 days.

Further, in an embodiment of the present invention,
5 cultivation is performed for 3 days to 7 days.

[0040]

In an embodiment of the present invention, this method further includes removing impurities before the nucleic acid is extracted in the step (b).

10 In the present invention, the impurities are, for example, substances other than bacteria or mycoplasmas, and specifically, are nucleic acids, proteins, and the like, and are preferably nucleic acid fragments of mycoplasmas.

In the present invention, as for removal of the
15 impurities, a method with which impurities decrease as compared with the case before removal is sufficient. Examples of the method of removing impurities include a method of filtering or collecting a specimen by a filter and a method of performing harvest by centrifugal
20 separation.

In the present invention, the impurities may pass through a filter so as to be removed or may remain on a filter without passing through a filter so as to be removed. Preferably, the impurities pass through a filter
25 so as to be removed.

In an embodiment of the present invention, removing of the impurities is performed by collecting the specimen with a filter.

[0041]

5 As for the filter for collection, a commercially available filter can be used. The pore size of the filter is, for example, 0.22 μm , 0.1 μm , 0.01 μm , and 0.005 μm . For example, in the case of a filter having a pore size of 0.1 μm , a substance having a size of about 0.1 μm , for
10 example, a virus or the like can be trapped. The filter may be a filter which is designed to exclude impurities having a specific molecular weight, and for example, the molecular weight cutoff (MWCO) of the filter is 1000 kDa, 100 kDa, and 50 kDa. For example, the filter having a
15 molecular weight cutoff of 100 kDa is a filter which can trap a substance having a size of about 100 kDa. In the present invention, the molecular weight cutoff of the filter is 50 to 1000 kDa and preferably 50 to 100 kDa.

 In the present invention, impurities may be smaller
20 than the molecular weight cutoff of the filter and be allowed to pass through the filter so as to be removed, or may be larger than the molecular weight cutoff of the filter and remain on the filter without being allowed to pass through the filter so as to be removed. Preferably,
25 impurities are smaller than the molecular weight cutoff of

the filter and pass through the filter.

In an embodiment of the present invention, a molecular weight cutoff of a filter is 1000 kDa or less or a pore size of a filter is 0.22 μm or less.

5 In an embodiment of the present invention, a molecular weight cutoff of a filter is 100 kDa or less.

[0042]

An aspect of the present invention relates to a method of managing quality of a biotechnology-related product, the
10 method including a step of testing presence or absence of a mycoplasma in a specimen by the method of the present invention.

An aspect of the present invention relates to a method of managing quality of a regenerative medicine-related
15 product, the method including a step of testing presence or absence of a mycoplasma in a specimen by the method of the present invention.

[0043]

An aspect of the present invention relates to a method
20 for testing presence or absence of a mycoplasma in a specimen, the method including:

(i) a step of removing impurities from a composition containing a specimen; and

(ii) a step of extracting a nucleic acid from the
25 composition containing a specimen from which impurities

have been removed and performing amplification of the nucleic acid using a primer capable of amplifying a nucleic acid sequence derived from a mycoplasma.

[0044]

5 In the present invention, the composition containing a specimen is, for example, a composition containing a culture medium, a reagent, or the like, which is used for testing presence or absence of a mycoplasma, in addition to a specimen.

10 In the present invention, impurities are, for example, substances other than bacteria or mycoplasmas, and specifically, are nucleic acids, proteins, and the like, and are preferably nucleic acid fragments of mycoplasmas.

 In the present invention, as for removal of the
15 impurities, a method with which impurities decrease as compared with the case before removal is sufficient. Examples of the method of removing impurities include a method of filtering or collecting a specimen by a filter and a method of performing harvest by centrifugal
20 separation.

 In the present invention, the impurities may pass through a filter so as to be removed or may remain on a filter without passing through a filter so as to be removed. Preferably, the impurities pass through a filter
25 so as to be removed.

In an embodiment of the present invention, in the step (i), the step of removing impurities is performed by collecting the specimen with a filter.

[0045]

5 As for the filter for collection, a commercially available filter can be used. The pore size of the filter is, for example, 0.22 μm , 0.1 μm , 0.01 μm , and 0.005 μm . For example, in the case of a filter having a pore size of 0.1 μm , a substance having a size of about 0.1 μm , for
10 example, a virus or the like can be trapped. The filter may be a filter which is designed to exclude impurities having a specific molecular weight, and for example, the molecular weight cutoff (MWCO) of the filter is 1000 kDa, 100 kDa, and 50 kDa. For example, the filter having a
15 molecular weight cutoff of 100 kDa is a filter which can trap a substance having a size of about 100 kDa. In the present invention, the molecular weight cutoff of the filter is 50 to 1000 kDa and preferably 50 to 100 kDa.

 In the present invention, impurities may be smaller
20 than the molecular weight cutoff of the filter and be allowed to pass through the filter so as to be removed, or may be larger than the molecular weight cutoff of the filter and remain on the filter without being allowed to pass through the filter so as to be removed. Preferably,
25 impurities are smaller than the molecular weight cutoff of

the filter and pass through the filter.

In an embodiment of the present invention, a molecular weight cutoff of a filter is 1000 kDa or less or a pore size of a filter is 0.22 μm or less.

5 In an embodiment of the present invention, a molecular weight cutoff of a filter is 100 kDa or less.

[0046]

In an embodiment of the present invention, this method further includes (iii) a step of performing electrophoresis
10 of an amplified product obtained from the step (ii).

In the present invention, the electrophoresis of the amplified product is not particularly limited as long as it is a method with which the amplified nucleic acid can be detected, and it is possible for those skilled in the art
15 to appropriately select an optimal method. For example, as for a method for preparing a gel such as agarose gel, selection of a buffer, and a method of checking a band, it is possible for those skilled in the art to appropriately select an optimal method. Examples of the method of
20 checking a band include staining of a nucleic acid with ethidium bromide, SYBR (registered trademark) Green, or the like and a southern blotting method.

[0047]

In the present invention, conditions of the cultivation
25 are not particularly limited as long as a method can

amplify a mycoplasma, and it is possible for those skilled in the art to appropriately select an optimal method. For example, types and composition of a culture medium, a cultivation temperature, an atmosphere, a CO₂ concentration, and the like can also be appropriately selected.

In the present invention, cultivation may be performed by aerobic cultivation or anaerobic cultivation and can be appropriately selected according to types of a mycoplasma to be tested.

For example, as for five kinds of *Mycoplasma arginini*, *Mycoplasma pneumoniae*, *Mycoplasma fermentans*, *Mycoplasma orale*, and *Acholeplasma laidlawii*, these mycoplasmas can be amplified under any of an aerobic condition and an anaerobic condition; however, since *Mycoplasma salivarium* can be amplified only under an anaerobic condition and *Mycoplasma hyorhinis* can be amplified only under an aerobic condition, six kinds other than *Mycoplasma salivarium* may be cultured under an aerobic condition and *Mycoplasma salivarium* may be cultured under an anaerobic condition.

In order to perform testing of all seven kinds, two or more specimens can be each cultured in an aerobic or anaerobic condition.

In an embodiment of the present invention, this method includes culturing the specimen before the step (ii).

In an embodiment of the present invention, this method includes culturing the specimen before the step (i).

[0048]

In the present invention, the period of time for
5 cultivation may be a period which is sufficient for
amplifying a mycoplasma to an amount beyond the detection
sensitivity obtained by this method, and can be arbitrarily
set. Non-limiting examples of the period of time for
cultivation include 1 day, 2 days, 3 days, 4 days, 5 days,
10 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days,
13 days, and 14 days. Non-limiting examples of the range
of the period of time for cultivation include 1 day to 14
days, 1 day to 7 days, 3 days to 14 days, 3 days to 7 days,
and 6 days to 10 days. The range thereof is preferably 3
15 days to 14 days and more preferably 6 days to 10 days.

[0049]

In an embodiment of the present invention, the specimen is a biotechnology-related product or a part thereof.

In an embodiment of the present invention, the specimen
20 is a regenerative medicine-related product or a part
thereof.

In an embodiment of the present invention, the
regenerative medicine-related product includes at least one
selected from a cell subjected to processing, a cell to be
25 provided to processing, a reagent to be used in processing

or testing, a culture medium for culturing a cell, a cell washing solution for washing a cell, and a preservation liquid for preserving a regenerative medicine product.

Specific examples thereof include a sheet-shaped cell

5 culture, a cell for producing a sheet-shaped cell culture (for example, a cryopreserved cell or the like), a sheet preparation culture medium, and a cell washing solution.

[0050]

An aspect of the present invention relates to a method
10 of managing quality of a biotechnology-related product, the method including a step of testing presence or absence of a mycoplasma in a specimen by the method of the present invention.

An aspect of the present invention relates to a method
15 of managing quality of a regenerative medicine-related product, the method including a step of testing presence or absence of a mycoplasma in a specimen by the method of the present invention.

[0051]

20 An aspect of the present invention relates to a kit for use in the method for testing presence or absence of a mycoplasma, the kit including a primer capable of amplifying a nucleic acid sequence derived from a mycoplasma.

25 The kit of the present invention includes, in addition

to the primer capable of amplifying a nucleic acid sequence derived from a mycoplasma, a culture medium for culturing a mycoplasma and/or a filter for removing impurities.

The kit of the present invention may further include,
5 for example, a tool for culturing a mycoplasma, a reagent or tool used in extraction of a nucleic acid, a reagent used in amplification of a nucleic acid, other tools, and instructions relating to a usage method.

[0052]

10 Examples of the tool for culturing a mycoplasma include a plate and a tube. Examples of the tool or reagent used in extraction of a nucleic acid include a reagent containing phenol, chloroform, isopropanol, ethanol, PEG, or the like, a column filled with silica beads or a silica
15 membrane, and an anion-exchange column. Examples of the reagent used in amplification of a nucleic acid include polymerase, dNTP, and a buffer. Examples of the other tools include a pipette, a dropper, tweezers, and a tube. Examples of the instructions relating to a usage method
20 include instructions for use and a medium on which information indicating a production method or a usage method, such as a flexible disk, a CD, a DVD, a Blu-ray Disc, a memory card, or an USB memory.

[0053]

25 In an embodiment of the present invention, the kit of

the present invention includes a culture medium for culturing a mycoplasma and a primer capable of amplifying a nucleic acid sequence derived from a mycoplasma.

In an embodiment of the present invention, the kit of
5 the present invention includes a primer capable of amplifying a nucleic acid sequence derived from a mycoplasma and a filter for removing impurities.

In an embodiment of the present invention, the kit of
10 the present invention includes a culture medium for culturing a mycoplasma, a primer capable of amplifying a nucleic acid sequence derived from a mycoplasma, and a filter for removing impurities.

Examples

[0054]

15 The present invention will be described in more detail with reference to the following examples; however, these are specific examples of the present invention and the present invention is not limited thereto.

[0055]

20 I. Material

1. Mycoplasma strain

The following seven kinds of mycoplasmas were used based on The Japanese Pharmacopoeia 17th Edition (JP17) General Information. Incidentally, all kinds thereof were
25 available from public storage institute and those which had

been passaged five times or less were used.

Mycoplasma arginini ATCC23838

Mycoplasma salivarium NBRC14478

Mycoplasma pneumoniae NBRC14401

5 *Mycoplasma fermentans* NBRC14854

Mycoplasma orale NBRC14477

Mycoplasma hyorhinis NBRC14858

Acholeplasma laidlawii NBRC14400

[0056]

10 2. Liquid culture medium for mycoplasma detection test

Heart Infusion Broth, glucose, arginine hydrochloride, and phenol red were mixed so as to be 18.75 g/L, 3.0 g/L, 3.0 g/L, and 0.025 g/L at a final concentration, respectively, and then autoclave sterilization was

15 performed at 121°C for 15 minutes. A horse serum, a yeast extract, and potassium penicillin G were added thereto so as to be 15%, 10%, and 250,000 units/L at a final concentration, respectively.

[0057]

20 3. Agar medium for mycoplasma amplification

Heart Infusion Broth and agar were mixed so as to be 17.5 g/L and 10 g/L at a final concentration, respectively, and then autoclave sterilization was performed at 121°C for 15 minutes. A horse serum and a yeast extract were added
25 thereto so as to be 20% and 10% at a final concentration,

respectively.

[0058]

4. Specimen

As for the specimen, cryopreserved cells, a sheet
5 preparation culture medium, and a cell washing solution
were used. The cryopreserved cells are cells obtained by
suspending skeletal myoblasts in a cryopreservation liquid
in a concentration of 3.0×10^7 cells/mL and then freezing
the suspended cells. In the following experiment,
10 cryopreserved cells, which were gently shaken up in a water
bath set at 37.0°C and then melted, were provided to the
test. The sheet preparation culture medium was obtained by
mixing a DMEM/F12 culture medium and human-derived serum in
an amount of 20% (v/v). The cell washing solution was
15 obtained by mixing a Hanks' buffered saline solution
(HBSS(-)) and human serum albumin in an amount of 0.5%
(v/v). Hereinafter, the "cryopreserved cells" are also
simply referred to as the "cells", the "sheet preparation
culture medium" is also simply referred to as the "culture
20 medium", and the "cell washing solution" is also simply
referred to as the "washing solution".

[0059]

II. Method and result

1. Setting of impurity removing step

25 100 μL of cells, which had been checked that live

mycoplasmas were not mixed, were added as a negative control to 2 mL of a liquid culture medium for a mycoplasma detection test, and then subjected to aerobic cultivation for 7 days under the conditions of 36°C and 5% CO₂.

5 Further, as a positive control, one obtained by adding 100 µL of cells and 10 µL of a mycoplasma diluent containing 7500 cfu/µL of *Acholeplasma laidlawii* to 2 mL of a liquid culture medium for a mycoplasma detection test was subjected to aerobic cultivation.

10 [0060]

DNA extraction was performed by using MagNA Pure LC DNA Isolation Kit-Large Volume (Roche) to finally obtain a 200 µL DNA extract. 90 µL of PCR reaction solution (Takara Ex taq) was added to 10 µL of the DNA extract and PCR was
 15 performed. As for PCR, PCR was performed for one cycle of 5 minutes at 40°C, one cycle of 10 minutes at 94°C, 20 cycles of 30 seconds at 94°C, 30 seconds at 70°C, and 45 seconds at 72°C (the part at 70°C decreases by 1°C per two cycles, up to 61°C), 25 cycles of 30 seconds at 94°C, 30
 20 seconds at 60°C, and 45 seconds at 72°C, and one cycle of 4 minutes at 72°C. As for the primer, the following sequences were used.

Primer sequences:

Forward: 5' -GGCGAATGGGTGAGTAACACG- 3'

25 Reverse: 5' -CGGATAACGCTTGCGACCTATG- 3'

The reaction solution after PCR was subjected to electrophoresis with an agarose gel, and then the presence or absence of a DNA band was checked.

[0061]

5 The result is shown in Fig. 1(A). M shows a 100 bp ladder, Lane 1 shows the result when PCR is performed without addition of a mycoplasma, and Lane 2 shows a positive control (the result when PCR is performed with addition of a mycoplasma). Even in a case where a
10 mycoplasma was not added (Lane 1), the band having the same size as that of a case where a mycoplasma is added (positive control, Lane 2) appeared. Since cells, which had been checked that live mycoplasmas were not mixed, were used as a specimen, it was considered that nucleic acid
15 fragments of the mycoplasma were mixed in this test system.

[0062]

In order to remove the nucleic acid fragments of the mycoplasma from the test system, trapping of the mycoplasma by a centrifugal ultrafiltration filter was examined.

20 100 μ L of cells, which had been checked that live mycoplasmas were not mixed, were added as a negative control to 2 mL of a liquid culture medium for a mycoplasma detection test, and then subjected to aerobic cultivation for 7 days under the conditions of 36°C and 5% CO₂.

25 Further, as a positive control, one obtained by adding 100

μL of cells and 10 μL of a mycoplasma diluent containing 7500 cfu/μL of *Acholeplasma laidlawii* to 2 mL of a liquid culture medium for a mycoplasma detection test was subjected to aerobic cultivation.

5 500 μL of the supernatant subjected to centrifugation for 5 minutes at 4°C and 400×g was transferred to a centrifugal ultrafiltration filter (Merck Millipore, Amicon Ultra 100 kDa) having a molecular weight cutoff of 100 kDa and then subjected to centrifugation for 10 minutes at 4°C
10 and 14,000×g. 500 μL of PBS was added thereto and subjected to centrifugation for 10 minutes at 4°C and 14,000×g, 500 μL of PBS was then further added and subjected to vigorously pipetting, and the solution was recovered. The reaction solution after PCR was subjected
15 to electrophoresis with an agarose gel and then the presence or absence of a DNA band was checked.

[0063]

The result is shown in Fig. 1(B). M shows a 100 bp ladder, Lanes 1 to 3 show the result when PCR is performed
20 without addition of a mycoplasma after collection with a filter, and Lanes 4 to 6 show a positive control (the result when PCR is performed with addition of a mycoplasma after collection with a filter). In the case of the negative control (Lanes 1 to 3), bands did not appear, and
25 only in a case where a mycoplasma was added (positive

control, Lanes 4 to 6), bands appeared. As described above, through the centrifugal ultrafiltration filter, it was shown that DNA fractions of the mycoplasma can be removed from the test system.

5 [0064]

2. Selection of filter

A centrifugal ultrafiltration filter for trapping a mycoplasma was examined. As for the centrifugal ultrafiltration filter, three types of a centrifugal
10 ultrafiltration filter having a molecular weight cutoff of 100 kDa (Merck Millipore, Amicon Ultra 100 kDa), a centrifugal ultrafiltration filter having a pore size of 0.1 μm (Merck Millipore, Ultrafree 0.1 μm), and a centrifugal ultrafiltration filter having a pore size of
15 0.22 μm (Merck Millipore, Ultrafree 0.22 μm) were used, and as for the mycoplasma, *Mycoplasma pneumoniae* and *Acholeplasma laidlawii* were used. As the specimen, a cell, a culture medium, and a washing solution were used. In a case where the specimen is a cell, 2000 μL of a liquid
20 culture medium for a mycoplasma detection test, 100 μL of a specimen, and 100 μL of 20000 cfu/mL mycoplasma diluent were added. 500 μL of the supernatant subjected to centrifugation for 5 minutes at 4°C and 400×g was transferred to a filter and then subjected to
25 centrifugation for 10 minutes at 4°C and 14,000×g. In a

case where the specimen is a culture medium or a washing solution, 2000 μL of a liquid culture medium for a mycoplasma detection test, 500 μL of a specimen, and 100 μL of 20000 cfu/mL mycoplasma diluent were added. 500 μL was transferred to a filter and then subjected to centrifugation for 10 minutes at 4°C and 14,000 $\times$ g. 500 μL of PBS was added thereto and subjected to centrifugation for 10 minutes at 4°C and 14,000 $\times$ g, 500 μL of PBS was then further added and subjected to vigorously pipetting, and the mycoplasma trapped with the filter was recovered. As a negative control, the same operation was performed in the condition without addition of a mycoplasma diluent.

[0065]

DNA was extracted from the filter-recovered liquid by using MagNA Pure LC DNA Isolation Kit-Large Volume (Roche) to finally obtain a 200 μL DNA extract.

10 μL of the DNA extract was added to 90 μL of PCR reaction solution (Takara Ex taq), and PCR was performed for one cycle of 5 minutes at 40°C, one cycle of 10 minutes at 94°C, 20 cycles of 30 seconds at 94°C, 30 seconds at 70°C, and 45 seconds at 72°C (the part at 70°C decreases by 1°C per two cycles, up to 61°C), 25 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 45 seconds at 72°C, and one cycle of 4 minutes at 72°C. As for the primer, the following sequences were used.

Primer sequences:

Forward: 5' -GGCGAATGGGTGAGTAACACG- 3'

Reverse: 5' -CGGATAACGCTTGCGACCTATG- 3'

The reaction solution after PCR was subjected to

5 electrophoresis with an agarose gel, and then the presence
or absence of a DNA band was checked. A case where a DNA
band of the mycoplasma can be confirmed in the filter-
recovered liquid was determined that the mycoplasma can be
trapped. The results are shown in Table 1. As for
10 *Mycoplasma pneumoniae*, the mycoplasma was able to be
trapped in any specimens with a filter having a pore size
of 0.22 μm . As for *Acholeplasma laidlawii*, the mycoplasma
was able to be trapped in the washing solution specimen
with a filter having a pore size of 0.22 μm , the mycoplasma
15 was able to be trapped in the culture medium specimen with
a filter having a pore size of 0.1 μm , and the mycoplasma
was able to be trapped in the cellular specimen with a
filter having a molecular weight cutoff of 100 kDa. In all
the specimens, only in the case of using a filter having a
20 molecular weight cutoff of 100 kDa, two kinds of
mycoplasmas were able to be trapped.

[0066]

[Table 1]

Table 1 Trapping possibility of mycoplasma in each filter

Filter	Specimen	Negative Control	Acholeplasma laidlawii	Mycoplasma pneumoniae
AmiconUltra 100kDa	Cell	-	+	+
	Culture medium	-	+	+
	Washing solution	-	+	+
Ultrafree 0.1 μ m	Cell	-	-	+
	Culture medium	-	+	+
	Washing solution	-	+	+
Ultrafree 0.22 μ m	Cell	-	+	+
	Culture medium	-	-	+
	Washing solution	-	+	+

+: trapping is possible, -: trapping is impossible

[0067]

3. Checking of proliferation of mycoplasma

5 As for seven kinds of mycoplasmas, it was checked how much the mycoplasma was amplified when the mycoplasma was added and cultured so as to have a concentration of 10 cfu/mL or less in the respective specimens of the cells, the culture medium, and the washing solution.

10 [0068]

In a case where the specimen is a cryopreserved cell, 2 mL of a liquid culture medium for a mycoplasma detection test, 100 μ L of a specimen, and 10 μ L of a mycoplasma diluent prepared to contain a desired mycoplasma were added to a well. The mycoplasma was added such that 0.80 cfu of the mycoplasma was contained in the case of testing *Mycoplasma arginini*, 0.54 cfu of the mycoplasma was

contained in the case of testing *Mycoplasma salivarium*,
0.68 cfu of the mycoplasma was contained in the case of
testing *Mycoplasma pneumoniae*, 0.60 cfu of the mycoplasma
was contained in the case of testing *Mycoplasma fermentans*,
5 0.60 cfu of the mycoplasma was contained in the case of
testing *Mycoplasma orale*, 0.74 cfu of the mycoplasma was
contained in the case of testing *Mycoplasma hyorhinis*, and
0.84 cfu the mycoplasma was contained in the case of
testing *Acholeplasma laidlawii*, per well. That is, the
10 mycoplasma was added such that the mycoplasma would be 0.80
cfu/100 μ L (8.0 cfu/mL) in the case of testing *Mycoplasma*
arginini, 0.54 cfu/100 μ L (5.4 cfu/mL) in the case of
testing *Mycoplasma salivarium*, 0.68 cfu/100 μ L (6.8 cfu/mL)
in the case of testing *Mycoplasma pneumoniae*, 0.60 cfu/100
15 μ L (6.0 cfu/mL) in the case of testing *Mycoplasma*
fermentans, 0.60 cfu/100 μ L (6.0 cfu/mL) in the case of
testing *Mycoplasma orale*, 0.74 cfu/100 μ L (7.4 cfu/mL) in
the case of testing *Mycoplasma hyorhinis*, and 0.84 cfu/100
 μ L (8.4 cfu/mL) in the case of testing *Acholeplasma*
20 *laidlawii*, per 100 μ L of the specimen.
[0069]

In a case where the specimen is a sheet preparation
culture medium or a cell washing solution, 2 mL of a liquid
culture medium for a mycoplasma detection test, 500 μ L of a
25 specimen, and 50 μ L of a mycoplasma diluent prepared to

contain a desired mycoplasma were added to a well. The mycoplasma was added such that 4.0 cfu of the mycoplasma was contained in the case of testing *Mycoplasma arginini*, 2.7 cfu of the mycoplasma was contained in the case of testing *Mycoplasma salivarium*, 3.4 cfu of the mycoplasma was contained in the case of testing *Mycoplasma pneumoniae*, 3.0 cfu of the mycoplasma was contained in the case of testing *Mycoplasma fermentans*, 3.0 cfu of the mycoplasma was contained in the case of testing *Mycoplasma orale*, 3.7 cfu of the mycoplasma was contained in the case of testing *Mycoplasma hyorhina*, and 4.2 cfu the mycoplasma was contained in the case of testing *Acholeplasma laidlawii*, per well. That is, the mycoplasma was added such that the mycoplasma would be 4.0 cfu/500 μ L (8.0 cfu/mL) in the case of testing *Mycoplasma arginini*, 2.7 cfu/500 μ L (5.4 cfu/mL) in the case of testing *Mycoplasma salivarium*, 3.4 cfu/500 μ L (6.8 cfu/mL) in the case of testing *Mycoplasma pneumoniae*, 3.0 cfu/500 μ L (6.0 cfu/mL) in the case of testing *Mycoplasma fermentans*, 3.0 cfu/500 μ L (6.0 cfu/mL) in the case of testing *Mycoplasma orale*, 3.7 cfu/500 μ L (7.4 cfu/mL) in the case of testing *Mycoplasma hyorhina*, and 4.2 cfu/500 μ L (8.4 cfu/mL) in the case of testing *Acholeplasma laidlawii*, per 500 μ L of the specimen.

[0070]

25 Six kinds of mycoplasmas excluding *Mycoplasma*

salivarium were subjected to aerobic cultivation under the conditions of 36°C and 5% CO₂, and Mycoplasma salivarium was subjected to anaerobic cultivation under the condition of 36°C. After 3 days from the cultivation, the culture solution was extracted from each well after 7 days, and the number of bacteria was measured.

The results are shown in Figs. 2 to 4. The amount of the mycoplasma after the cultivation for 7 days was increased to 1500 to 10⁹ cfu/mL although varying depending on the type of bacteria, and in this precultivation step, it was confirmed that a small amount of the mycoplasma is increased to a sufficient amount.

[0071]

4. Calculation of detection limit

In respective specimens of the cells, the culture medium, and the washing solution, detection limits of seven kinds of mycoplasmas were calculated.

[0072]

In a case where the specimen is a cryopreserved cell, 852 µL of a liquid culture medium for a mycoplasma detection test and 48 µL of a specimen were added to a microtube, and 100 µL of a mycoplasma diluent prepared to contain a desired mycoplasma was added thereto. The mycoplasma was added so as to be 1000 cfu, 500 cfu, 100 cfu, and 50 cfu per microtube. That is, with respect to

the total volume in the microtube, the mycoplasma was added so as to have concentrations of 1000 cfu/mL, 500 cfu/mL, 100 cfu/mL, and 50 cfu/mL.

[0073]

5 In a case where the specimen is a sheet preparation culture medium or a cell washing solution, 525 μ L of a liquid culture medium for a mycoplasma detection test, 150 μ L of a specimen, and 75 μ L of a mycoplasma diluent prepared to contain a desired mycoplasma were added to a
10 microtube. The mycoplasma was added so as to be 750 cfu, 375 cfu, 75 cfu, and 37.5 cfu per microtube. That is, with respect to the total volume in the microtube, the mycoplasma was added so as to have concentrations of 1000 cfu/mL, 500 cfu/mL, 100 cfu/mL, and 50 cfu/mL.

15 [0074]

In a case where the specimen is a cryopreserved cell, 500 μ L of the supernatant subjected to centrifugation for 5 minutes at 4°C and 400×g was transferred to a centrifugal ultrafiltration filter (Merck Millipore) having a molecular
20 weight cutoff of 100 kDa and then subjected to centrifugation for 10 minutes at 4°C and 14,000×g.

In a case where the specimen is a culture medium or a washing solution, 500 μ L was transferred to a centrifugal ultrafiltration filter (Merck Millipore) having a molecular
25 weight cutoff of 100 kDa and then subjected to

centrifugation for 10 minutes at 4°C and 14,000×g. 500 µL of PBS was added thereto and subjected to centrifugation for 10 minutes at 4°C and 14,000×g, 500 µL of PBS was then further added and subjected to vigorously pipetting, and
5 the mycoplasma trapped with the filter was recovered.

[0075]

DNA was extracted from the total amount of the recovered liquid after removing impurities by using MagNA Pure LC DNA Isolation Kit-Large Volume (Roche) to finally
10 obtain a 200 µL DNA extract.

[0076]

10 µL of the DNA extract was added to 90 µL of PCR reaction solution (Takara Ex taq), and PCR was performed for one cycle of 5 minutes at 40°C, one cycle of 10 minutes
15 at 94°C, 20 cycles of 30 seconds at 94°C, 30 seconds at 70°C, and 45 seconds at 72°C (the part at 70°C decreases by 1°C per two cycles, up to 61°C), 25 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 45 seconds at 72°C, and one
20 cycle of 4 minutes at 72°C. As for the primer, the following sequences were used.

Primer sequences:

Forward: 5' -GGCGAATGGGTGAGTAACACG- 3'

Reverse: 5' -CGGATAACGCTTGCGACCTATG- 3'

The reaction solution after PCR was subjected to
25 electrophoresis with an agarose gel, and then the presence

or absence of a DNA band was checked.

[0077]

Calculation was performed 24 times for respective mycoplasma concentrations of 1000 cfu/mL, 500 cfu/mL, 100
 5 cfu/mL, and 50 cfu/mL, and from the ratio of the mycoplasma concentration and the number of positive cells (the number of cells in which a DNA band specific to the mycoplasma was confirmed), probit analysis was performed using NATIONAL INSTITUTE OF INFECTIOUS DISEASE statistical analysis
 10 support software Bioassay Assist, and 95% detection sensitivity was calculated. The results are shown in Table 2. The detection limit of each mycoplasma was 92 to 5647 cfu/mL.

[0078]

15 [Table 2]

Table 2 Detection limit

Specimen	M. arginini	M. salivarium	M. pneumoniae	M. fermentans
Cell	132 cfu/mL	202 cfu/mL	1043 cfu/mL	176 cfu/mL
Culture medium	335 cfu/mL	474 cfu/mL	542 cfu/mL	124 cfu/mL
Washing solution	473 cfu/mL	104 cfu/mL	502 cfu/mL	92 cfu/mL
Specimen	M. orale	M. hyorhinis	A. laidlawii	
Cell	1365 cfu/mL	1068 cfu/mL	3044 cfu/mL	
Culture medium	751 cfu/mL	1091 cfu/mL	2579 cfu/mL	
Washing solution	633 cfu/mL	544 cfu/mL	5647 cfu/mL	

[0079]

5. Detectability of mycoplasma

In the respective specimens of the cells, the culture medium, and the washing solution, the amount of the mycoplasma amplified in the precultivation was compared with the detection sensitivity in this method, and it was
5 verified whether the mycoplasma contamination can be detected with a high sensitivity as the final test method. The amount of the mycoplasma amplified in the precultivation and the detection sensitivity in this method are shown in Table 3 and Figs. 5 to 7. In all the seven
10 kinds of mycoplasmas, the amount of the mycoplasma amplified in the precultivation which was calculated in 3. exceeded the detection sensitivity calculated in 4. Therefore, in all the seven kinds of mycoplasmas, it was verified that the mycoplasma can be detected with a
15 sensitivity of 10 cfu/mL or less from 100 μ L of the specimen containing 1 cfu or less of a mycoplasma in the case of the cellular specimen and from 500 μ L of the specimen containing 5 cfu or less of a mycoplasma in the case of the culture medium and the washing solution
20 specimens.

[0080]

[Table 3]

Table 3 Detectability of mycoplasma

Specimen	Mycoplasma	Mycoplasma contamination per 1 mL of specimen (cfu/mL)	Culture solution after precultivation (A) (cuf/mL)	Detection sensitivity (B) (cfu/mL)	Detecting possibility (possible: A > B) (impossible: A < B)
Cell	M. arginini	8.0	5.79×10^9	132	Possible
	M. salivarium	5.4	3.53×10^8	202	Possible
	M. pneumoniae	6.8	1.54×10^3	1043	Possible
	M. fermentans	6.0	5.43×10^5	176	Possible
	M. orale	6.0	1.17×10^9	1365	Possible
	M. hyorhinis	7.4	1.58×10^8	1068	Possible
	A. laidlawii	8.4	3.62×10^7	3044	Possible
Culture medium	M. arginini	8.0	1.00×10^9	335	Possible
	M. salivarium	5.4	6.18×10^7	474	Possible
	M. pneumoniae	6.8	6.77×10^3	542	Possible
	M. fermentans	6.0	1.24×10^8	124	Possible
	M. orale	6.0	2.56×10^7	751	Possible
	M. hyorhinis	7.4	6.20×10^7	1091	Possible
	A. laidlawii	8.4	4.98×10^7	2579	Possible
Washing solution	M. arginini	8.0	4.82×10^8	473	Possible
	M. salivarium	5.4	6.22×10^7	104	Possible
	M. pneumoniae	6.8	4.85×10^3	502	Possible
	M. fermentans	6.0	3.38×10^8	92	Possible
	M. orale	6.0	2.71×10^8	633	Possible
	M. hyorhinis	7.4	1.84×10^7	544	Possible
	A. laidlawii	8.4	1.44×10^8	5647	Possible

[0081]

6. Mycoplasma detection test

5 6.1. Precultivation

500 μ L of a washing solution was added as a specimen to 2 mL of a liquid culture medium for a mycoplasma detection test and then subjected to aerobic cultivation for 7 days under the conditions of 36°C and 5% CO₂. Similarly, 500 μ L of a washing solution was added as a specimen to 2 mL of a liquid culture medium for a mycoplasma detection test and then subjected to anaerobic cultivation for 7 days under

the condition of 36°C. As for a negative control, PBS was added instead of a specimen and the same operation was performed. Further, as a positive control, one obtained by adding 50 µL of a mycoplasma diluent containing 1500 cfu/µL of *Mycoplasma orale* was subjected to aerobic cultivation, and one obtained by adding 50 µL of a mycoplasma diluent containing 1500 cfu/µL of *Mycoplasma salivarium* was subjected to anaerobic cultivation.

6.2. Impurity removal

500 µL of a culture solution was transferred to a centrifugal ultrafiltration filter (Merck Millipore) having a molecular weight cutoff of 100 kDa and then subjected to centrifugation for 10 minutes at 4°C and 14,000×g. 500 µL of PBS was added thereto and subjected to centrifugation for 10 minutes at 4°C and 14,000×g, 500 µL of PBS was then further added and subjected to vigorously pipetting, and the mycoplasma trapped with the filter was recovered.

[0082]

6.3. DNA extraction

DNA was extracted from the total amount of the recovered liquid after removing impurities by using MagNA Pure LC DNA Isolation Kit-Large Volume (Roche) to finally obtain a 200 µL DNA extract.

6.4. PCR and electrophoresis

10 µL of the DNA extract was added to 90 µL of PCR

reaction solution (Takara Ex taq), and PCR was performed for one cycle of 5 minutes at 40°C, one cycle of 10 minutes at 94°C, 20 cycles of 30 seconds at 94°C, 30 seconds at 70°C, and 45 seconds at 72°C (the part at 70°C decreases by 1°C per two cycles, up to 61°C), 25 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 45 seconds at 72°C, and one cycle of 4 minutes at 72°C. As for the primer, the following sequences were used.

Primer sequences:

10 Forward: 5' -GGCGAATGGGTGAGTAACACG- 3'

Reverse: 5' -CGGATAACGCTTGCGACCTATG- 3'

The reaction solution after PCR was subjected to electrophoresis with an agarose gel, and then the presence or absence of a DNA band was checked.

15 6.5. Result

The result is shown in Fig. 8. M shows a 100 bp ladder, Lane 1 shows a negative control (the result when PCR is performed with respect to water), Lanes 2 to 4 show a negative control (the result when PCR is performed by aerobic cultivation using PBS instead of a specimen), Lanes 5 to 7 show the result when PCR is performed by aerobic cultivation of a specimen, Lanes 8 to 10 show a positive control (the result when PCR is performed by aerobic cultivation with addition of a mycoplasma diluent (Mycoplasma orale) to a specimen), Lanes 11 to 13 show a

negative control (the result when PCR is performed by anaerobic cultivation using PBS instead of a specimen), Lanes 14 to 16 show the result when PCR is performed by anaerobic cultivation of a specimen, and Lanes 17 to 19 show a positive control (the result when PCR is performed by anaerobic cultivation with addition of a mycoplasma diluent (*Mycoplasma salivarium*) to a specimen). Bands appeared in Lanes 8 to 10 and 17 to 19 as positive controls, and bands did not appear in Lanes 2 to 4 and 11 to 13 as negative controls. Since bands did not appear even in Lanes 5 to 7 and 14 to 16 in which the specimen flowed, it was checked that the mycoplasma did not exist in the specimen.

[0083]

Various characteristics of the present invention described in this specification can be combined in various ways, and embodiments obtained by such combinations, inclusive of combinations not specifically described in this specification, are all within the scope of the present invention. In addition, persons skilled in the art understand that a multiplicity of various modifications are possible without departing from the spirit of the present invention, and equivalents including such modifications are also included within the scope of the present invention. Therefore, it should be understood that the embodiments

described in this specification are mere exemplifications and are not described with an intention to limit the scope of the present invention.

CLAIMS

1. A method for testing presence or absence of a mycoplasma in a specimen, the method comprising:

(a) a step of culturing a specimen; and

(b) a step of extracting a nucleic acid from the cultured specimen and performing amplification of the nucleic acid using a primer capable of amplifying a nucleic acid sequence derived from a mycoplasma,

wherein the specimen is a biotechnology-related product or a part thereof.

2. The method according to claim 1, wherein the specimen is a regenerative medicine-related product or a part thereof.

3. The method according to claim 1 or 2, wherein the step of performing amplification of the nucleic acid is performed by a polymerase chain reaction (PCR) method.

4. The method according to any one of claims 1 to 3, further comprising (c) a step of performing electrophoresis of an amplified product obtained from the step (b).

5. The method according to any one of claims 1 to 4, wherein the method includes performing anaerobic cultivation in the step (a).

6. The method according to any one of claims 1 to 4, wherein in the step (a), two or more specimens are each cultured in an aerobic or anaerobic condition.

7. The method according to any one of claims 1 to 6,
wherein an amount of the specimen cultured in the step (a)
is 10 μ L to 10 mL.

8. The method according to any one of claims 1 to 7,
5 wherein in the step (a), cultivation is performed for 3 to
14 days.

9. The method according to any one of claims 1 to 8,
further comprising removing impurities before the nucleic
acid is extracted in the step (b).

10 10. The method according to any one of claims 1 to 9,
wherein removing of impurities is performed by collecting
the specimen with a filter.

11. The method according to any one of claims 1 to 10,
wherein a molecular weight cutoff of a filter is 1000 kDa
15 or less or a pore size of a filter is 0.22 μ m or less.

12. The method according to any one of claims 1 to 11,
wherein the regenerative medicine-related product includes
at least one selected from a cell subjected to processing,
a cell to be provided to processing, a reagent to be used
20 in processing or testing, a culture medium for culturing a
cell, a cell washing solution for washing a cell, and a
preservation liquid for preserving a regenerative medicine
product.

13. A method of managing quality of a biotechnology-related
25 product, the method comprising:

a step of testing presence or absence of a mycoplasma in a specimen by the method according to any one of claims 1 to 12.

14. A kit for use in the method according to any one of
5 claims 1 to 12, the kit comprising: a culture medium for culturing a mycoplasma; and a primer capable of amplifying a nucleic acid sequence derived from mycoplasma.

15. The kit according to claim 14, further comprising a filter for removing impurities.