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A METHOD OF DIAGNOSIS AND AGENTS USEFUL FOR SAME ✓

FIELD OF THE INVENTION

- 5 The present invention relates generally to a method of diagnosis and agents useful for same. More particularly, the present invention provides for a method of diagnosing or confirming the diagnosis of genital ulceration or other diseases caused or exacerbated by *Calymmatobacterium* species or related organisms.

10 BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

- 15 The rapidly increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical and allied health fields. This is particularly the case in the area of diagnosis. DNA fingerprints have the potential to provide a highly sensitive and rational approach to medical diagnosis, especially for infections where the causative organism cannot be cultured and/or diagnosis is dependent on techniques of low sensitivity such as
20 histology.

- Granuloma inguinale, or donovanosis as it is more commonly known, is a disease condition resulting in genital ulceration and other systemic illness. The disease is a cause of considerable morbidity. In addition, it may increase the risk of the transmission of human immunodeficiency
25 virus (HIV) and, hence, the rapid diagnosis and treatment of donovanosis is becoming an important priority in the control of sexually transmitted diseases.

- The diagnosis of donovanosis has previously been problematic and has generally relied on the detection of typical histological features in smears and biopsies. The sensitivity of these
30 histological techniques varies from between 60 and 80% depending on the ulcer type, the stain used and the experience and diligence of the histopathologist (1, 2). There is a need, therefore,

to develop a diagnostic procedure which is both convenient and highly sensitive.

The causative organism of donovanosis is *Calymmatobacterium granulomatis*. This organism cannot be cultured in conventional microbiological media although it can be maintained following inoculation in the yolk sac of chick embryos (1, 3, 4). Although culture of the organism has been reported in human monocytes (11) and Hep-2 cells (12), difficulties in culturing the organism have hampered the development of diagnostic techniques for *C. granulomatis*. In any event, reliance on the culturing of highly fastidious infectious agents as a form of diagnosis is expensive and time consuming and requires adequate and careful transport from the patient to an appropriate reference laboratory.

In work leading up to the present invention, the inventors sought a nucleic acid based assay for the causative organism of donovanosis. A nucleic acid based assay has many advantages; these include sensitivity, no reliance on the maintenance of viable organisms and the fact that there is a lesser probability of loss of diagnostic yield if there is a delay between collection and assay.

SUMMARY OF THE INVENTION

Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide sequences referred to herein are defined after the bibliography.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

One aspect of the present invention contemplates a method of detecting *Calymmatobacterium* species in a sample said method comprising detecting the presence or absence of a nucleotide sequence of said *Calymmatobacterium* species or an organism related thereto wherein the presence or absence of said nucleotide sequence is indicative of *Calymmatobacterium* species in the sample.

Another aspect of the present invention provides a method of identifying *C. granulomatis* in a sample said method comprising detecting the presence of a nucleotide sequence from *C. granulomatis* or an organism related thereto wherein the identification of said nucleotide sequence is indicative of *C. granulomatis* in said sample.

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Yet another aspect of the present invention contemplates a method of detecting *C. granulomatis* in a sample said method comprising detecting the presence of a nucleotide sequence from *C. granulomatis* or an organism related thereto with a nucleic acid molecule derived from an organism genetically related to *C. granulomatis* wherein the presence of said nucleotide sequence
10 it indicative of *C. granulomatis* in the sample.

Still yet another aspect of the present invention, there is provided a method of detecting *C. granulomatis* in a sample said method comprising detecting the presence of a first nucleotide sequence with a heterologous nucleic acid molecule from an organism other than *C. granulomatis* wherein first nucleotide sequence is substantially represented in both said organism
15 and *C. granulomatis* and then determining the presence or absence of a second nucleotide sequence which is absent from *C. granulomatis* but present in said organism using another heterologous nucleic acid molecule, wherein the presence of said first nucleotide sequence and absence of said second nucleotide sequence is indicative of *C. granulomatis* in said sample.

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Even yet a further aspect of the present invention contemplates a method of detecting *C. granulomatis* in a sample, said method comprising determining the presence of a *phoE* related sequence in said sample using a nucleic acid probe derived from *Klebsiella phoE* and determining the absence of *scrA* related sequences using a nucleic acid probe derived from *Klebsiella scrA*
25 wherein the presence of *phoE* related sequences but absence of *scrA* related sequences in said sample is indicative of the presence of *C. granulomatis*.

Still in yet a further aspect of the present invention, there is contemplated a method of detecting *C. granulomatis* in a sample, said method comprising isolating genetic sequences having a high
30 degree of nucleotide similarity from *C. granulomatis* and a species of *Klebsiella*, digesting said genetic sequences with a restriction endonuclease which cleaves the genetic sequence from *C.*

granulomatis resulting in a restriction pattern different to the pattern obtained when the same enzyme cleaves the genetic sequence from a *Klebsiella* species wherein the presence of a particular restriction endonuclease profile is indicative of whether the sample contains *C. granulomatis* or a *Klebsiella* species or neither organism.

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Another aspect of the present invention contemplates a method for diagnosing donovanosis, said method comprising collecting a sample from a lesion and determining the presence of a genetic sequence substantially unique to *C. granulomatis* wherein the presence of such sequences is indicative of donovanosis.

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A further aspect of the present invention provides a method of diagnosing donovanosis in a subject with genital ulceration, said method comprising obtaining a sample from a lesion or ulcer from the genital region, subjecting said sample to PCR using a nucleic acid probe capable of amplifying *C. granulomatis phoE* and then subjecting the sample to restriction digestion wherein
15 the pattern of the restriction digestion determines whether the sample contains *C. granulomatis* *Klebsiella*.

BRIEF DESCRIPTION OF THE FIGURES

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Figure 1 is a schematic representation of the sucrose regulon in *Klebsiella* species.

Figure 2 is a photographic representation showing PCR products derived from DNA templates from control organisms, original swab specimens and culture specimens. Lane 1: molecular
25 weight markers; lane 2: 382 bp fragment (*phoE*) from *Klebsiella pneumoniae*; lane 3: 438 bp fragment (*scrA*) from *K. pneumoniae*; lane 4: *phoE* from original swab, case 1; lane 5: *scrA* from original swab, case 1; lane 6: *phoE* from culture, case 1; lane 7: *scrA* from culture, case 1.

Figure 3 is a photographic representation showing ethidium bromide stained agarose gel of
30 amplification products obtained from targeting the sucrose regulon of *Klebsiella*. Lanes 1 and 14, PhiX174 *HaeIII* markers (New England Biolabs, Inc.); Lanes 2, 6, 10, *K. pneumoniae*;

Lanes 3, 7, 11, *K. ozaenae*; Lanes 4, 8, 12, *K. rhinoscleromatis*; Lanes 5, 9, 13, *C. granulomatis*. Expected product sizes for primer sets F3/R1 (lanes 205, F4/R4 (lanes 6-9) and F5/R5 (lanes 10-13) are 272 bp, 438 bp and 650 bp, respectively.

5 Figure 4 is a photographic representation showing ethidium bromide stained agarose gel of amplification products obtained for *K. pneumoniae* (lanes 2-3), *K. ozaenae* (lanes 4-5), *K. rhinoscleromatis* (lanes 6-7), clinical donovanosis punch biopsies x 2 (lanes 8-11), clinical donovanosis swabs x 2 (lanes 12-15), clinical syphilis swab (lanes 16-17) and clinical herpes genitalis swab (lanes 18-19) when using the *phoE* and *scrA* primer sets (A and B). Primers
10 targeting *phoE* (382bp product) were used in lanes 2, 4, 6, 8, 10, 12, 14, 16 and 18. Primers targeting *scrA* (468bp product) were used in lanes 3, 5, 7, 9, 11, 13, 15, 17 and 19. Lanes 1 and 20 contain PhiX174 *Hae*III markers (New England Biolabs, Inc.).

Figure 5 is a schematic representation of the 700bp *phoE* product amplified with primer set
15 *phoEdig1*. The top arrows represent *Hae*III restriction sites for the *C. granulomatis* derived product and the bottom arrows represent *Hae*III restriction sites for products derived from *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis*. Numbers represent fragment lengths in base pairs.

20 Figure 6 is a photographic representation of ethidium bromide stained agarose gel of *Hae*III digested products amplified with the *phoEdig1* primer set (700bp). The bands marked as ~40 bp consist of products ranging from 33bp-45bp. The band marked as 87 bp also consists of an 86 bp band for the *Klebsiella*. Lanes 1 and 6 -PhiX174 *Hae*III markers (New England Biolabs, Inc.); Lane 2- *K. pneumoniae*; Lane 3 - *K. ozaenae*; Lane 4 - *K. rhinoscleromatis*; Lane 5 -
25 clinical specimen.

Figure 7 is diagrammatic representation showing use of a labelled *phoE* fragment, *Hae*III digest and magnetic beads in an *in vitro* assay for *C. granulomatis*. Kpn, *K. pneumoniae*; Koz, *K. ozaenae*; Krh, *K. rhinoscleromatis*; Cgr, *C. granulomatis*; 1, *Hae*III restriction sites; capture
30 site; 9, capture oligonucleotide attracted to magnetic bead.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to the detection of disease conditions caused or exacerbated by *Calymmatobacterium* species. The present invention further provides a means of
5 distinguishing between *Calymmatobacterium* species and *Klebsiella* species.

Accordingly, one aspect of the present invention contemplates a method of detecting *Calymmatobacterium* species in a sample said method comprising detecting the presence or absence of a nucleotide sequence of said *Calymmatobacterium* species or an organism related
10 thereto wherein the presence or absence of said nucleotide sequence is indicative of *Calymmatobacterium* species in the sample.

To date, the only species in the genus *Calymmatobacterium* is *C. granulomatis*. Accordingly, the present invention is particularly directed to the identification of *C. granulomatis*. This is
15 done, however, with the understanding that the present invention extends to new species of the genus *Calymmatobacterium* if subsequently identified. Furthermore, the present invention extends to organisms related to *C. granulomatis* at the nucleic acid, physiological, structural, immunological, biochemical or clinical levels. Reference hereinafter to "*C. granulomatis*" includes reference to other species of *Calymmatobacterium* as well as organisms related to *C.*
20 *granulomatis*.

Accordingly, another aspect of the present invention provides a method of identifying *C. granulomatis* in a sample said method comprising detecting the presence or absence of a nucleotide sequence from *C. granulomatis* or an organism related thereto wherein the
25 identification of the presence or absence of said nucleotide sequence is indicative of *C. granulomatis* in said sample.

The sample is generally a specimen taken from a lesion, and more particularly, a granulomatis lesion or ulcer, from the genital region or surrounding area. A specimen may, for example, be
30 a smear, biopsy, exudated fluid, tissue sample, pustulate fluid or other source where the causative organism or its nucleic acid is likely to be located. Conveniently, a tissue sample comprising

mononuclear cells is used. Smears stained with, for example, Wright's stain generally show the *C. granulomatis* pathogen in clusters of blue or black "Donovan bodies" in the cytoplasm of large mononuclear cells. The presence of Donovan bodies, however, is not solely indicative of infection by *C. granulomatis*.

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The detection of a nucleotide sequence indicative of *C. granulomatis* may be conducted in a number of ways including gel based assays or use of specific primers. For example, a nucleic acid probe or primer interactive with *C. granulomatis* genetic sequences may be employed in techniques such as polymerase chain reaction (PCR) and hybridization. Other techniques include
10 restriction fragment length polymorphism (RFLP), and single strand conformation polymorphism (SSCP) amongst many other techniques. The nucleic acid probe or primer may be specific for *C. granulomatis* or may be interactive against *C. granulomatis* and an organism related thereto.

As stated above, a nucleic acid probe may be selected from an organism having at least some
15 genetic similarity to a genetic sequence in *C. granulomatis*. One particularly useful organism in this regard is a species of the genus *Klebsiella*. Some members of this genus have a common ultrastructural morphology (5) and antigenic crossreactivity (6) with *C. granulomatis*. Examples of *Klebsiella* genes which have substantial similarity to genetic sequences in *C. granulomatis* include phosphate porin (*phoE*), 16S ribosomal RNA, glutamate dehydrogenase (*gdhA*) and
20 acetolactate synthase (*ilvK*). In accordance with a preferred aspect of the present invention, therefore, the presence of *C. granulomatis* is detected using a nucleic acid molecule derived from another organism such as but not limited to *Klebsiella* species.

Accordingly, another aspect of the present invention contemplates a method of detecting *C.*
25 *granulomatis* in a sample said method comprising detecting the presence of a nucleotide sequence from *C. granulomatis* with a nucleic acid molecule derived from an organism genetically related to *C. granulomatis* wherein the presence of said nucleotide sequence is indicative of *C. granulomatis* in the sample.

30 Preferably, the nucleic acid molecule is from *Klebsiella* species such as but not limited to *Klebsiella pneumoniae*, *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis*.

The nucleic acid molecule from an organism other than *C. granulomatis* is referred to as a "heterologous" nucleic acid molecule.

Reference herein to a heterologous nucleic acid molecule includes but is not limited to a nucleic acid probe or primer, an oligonucleotide, a non-full length gene or a full length gene. The nucleic acid may be DNA, genomic DNA, cDNA, plasmid DNA or mRNA. It may also be single or double stranded. Similarly, the target *C. granulomatis* nucleotide sequence may be a coding or non-coding region, a structural or regulatory gene and DNA, cDNA, plasmid DNA or mRNA.

10 Where a heterologous nucleic acid molecule is used to detect the presence of *C. granulomatis* nucleotide sequences, it may be necessary to include a control to distinguish between *C. granulomatis* and the organism from which the nucleic acid probe is derived. This may be accomplished in any number of ways including confirmational culturing, histological examination, microscopic examination, immunological means or by genetic means. The latter is preferred due
15 to its rapidity and sensitivity.

In a particularly preferred embodiment, a first heterologous nucleic acid probe is used which is capable of identifying a nucleotide sequence for *C. granulomatis* and a related organism such as *Klebsiella* and a second heterologous nucleic acid probe is then used which will not detect *C. granulomatis* nucleotide sequences but would detect an organism related thereto. For example,
20 *C. granulomatis* lacks the sucrose regulon which is present in *Klebsiella* species. However, the *Klebsiella phoE* gene is highly conserved in *C. granulomatis*. This enables a differential screening protocol where the presence of *phoE* related genetic sequences is first identified using a nucleic acid probe derived from *Klebsiella phoE* and then a probe from a gene from the
25 *Klebsiella* sucrose regulon, eg. *scrA* (see Figure 1), is used to determine whether the detected *phoE* is from *Klebsiella* or *C. granulomatis*.

According to this aspect of the present invention, there is provided a method of detecting *C. granulomatis* in a sample said method comprising detecting the presence of a first nucleotide
30 sequence with a nucleic acid molecule from an organism other than *C. granulomatis* wherein first nucleotide sequence is substantially represented in both said organism and *C. granulomatis* and

then determining the presence or absence of a second nucleotide sequence which is absent from *C. granulomatis* but present in said organism using another nucleic acid molecule, wherein the presence of said first nucleotide sequence and absence of said second nucleotide sequence is indicative of *C. granulomatis* in said sample.

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In one embodiment, the first nucleotide sequence detected is related to *Klebsiella phoE*.

In a related embodiment, the second nucleotide sequence is related to *Klebsiella scrA*.

- 10 According to a particularly preferred embodiment, there is provided a method of detecting *C. granulomatis* in a sample, said method comprising determining the presence of a *phoE* related sequence in said sample using a nucleic acid probe derived from *Klebsiella phoE* and determining the absence of *scrA* related sequences using a nucleic acid probe derived from *Klebsiella scrA* wherein the presence of *phoE* related sequences but absence of *scrA* related sequences in said
15 sample is indicative of the presence of *C. granulomatis*.

The differential screening procedure may be employed with any number of *Klebsiella* genetic sequences provided that there are genetic sequences in common between both organisms and there are also genetic sequences substantially unique to either *Klebsiella* species or *C. granulomatis*.
20

Another convenient procedure exploits base changes in conserved genes in both *C. granulomatis* and *Klebsiella* species. In particular, a comparison of genes having a high degree of nucleotide sequence similarity in *C. granulomatis* and in *Klebsiella* sp, can identify differences detectable
25 at the restriction endonuclease profile level.

According to this aspect of the present invention, there is contemplated a method of detecting *C. granulomatis* in a sample, said method comprising isolating genetic sequences having a high degree of nucleotide similarity from *C. granulomatis* and a species of *Klebsiella*, digesting said
30 genetic sequences with a restriction endonuclease which cleaves the genetic sequence from *C. granulomatis* resulting in a restriction pattern different to the pattern obtained when the same

enzyme cleaves the genetic sequence from a *Klebsiella* species wherein the presence of a particular restriction endonuclease profile is indicative of whether the sample contains *C. granulomatis* or a *Klebsiella* species or neither organism.

- 5 This aspect of the present invention is particularly exemplified herein with respect to the nucleotide sequence difference in the *phoE* genes in *C. granulomatis* and *Klebsiella* species. The former *phoE* gene carries two base changes unique to *C. granulomatis*. The changes correspond to position 502 of the coding sequence (C → T) which results in an Arg → Cys change and
.. position 584 of the coding sequence (G → A) which results in a silent change. These changes
10 also result in elimination of *Hae*II restriction sites. Accordingly, a *phoE* primer is used to optionally amplify the *phoE* genetic sequences from *C. granulomatis* and *Klebsiella* species and then these sequences are subjected to *Hae*II cleavage or cleavage with another suitable enzyme to detect the presence or absence of *Hae*II fragments. The presence of a larger *Hae*II fragment is indicative of *C. granulomatis* whereas the absence of this fragment indicates the presence of
15 *Klebsiella* species.

A combination of both the *phoE/scrA* differential assay and the PCR/restriction digestion assay provides, therefore, even greater sensitivity for the detection of *C. granulomatis*.

- 20 The detection of *C. granulomatis* is particularly useful in diagnosing, or confirming the diagnosis of, donovanosis.

Accordingly, another aspect of the present invention contemplates a method for diagnosing donovanosis, said method comprising collecting a sample from a lesion and determining the
25 presence of a genetic sequence substantially unique to *C. granulomatis* and/or absent from *C. granulomatis* wherein the presence or absence of such sequences is indicative of donovanosis.

- More particularly, the present invention is directed to a method of diagnosing donovanosis in a subject with genital ulceration, said method comprising obtaining a sample from a lesion or ulcer
30 from the genital or extra-genital area, subjecting said sample to PCR using a nucleic acid probe or primer derived from *Klebsiella phoE* and then subjecting the sample to PCR using a nucleic

acid probe or primer derived from *Klebsiella scrA* wherein the presence of *phoE* related genetic sequences but absence of *scrA* genetic sequences is indicative of donovanosis. An extra-genital area includes mouth, bones, abdomen, mucous surfaces, the anal rectal area amongst other areas.

5

In a related embodiment, the present invention provides a method of diagnosing donovanosis in a subject with genital ulceration, said method comprising obtaining a sample from a lesion or ulcer from the genital or extra-genital area, subjecting said sample to PCR using a nucleic acid probe or primer capable of amplifying *C. granulomatis phoE* and then subjecting the sample to
10 restriction digestion wherein the pattern of restriction digestion determines whether the sample contains *C. granulomatis* or a related organism such as *Klebsiella*.

A combination assay is also contemplated by the present invention involving the differential PCR assay for *phoE/scrA* and the PCR/restriction assay for *phoE/HaeII*. These two assays may be
15 conducted in either order or may be conducted simultaneously. Although a PCR procedure to amplify *phoE* prior to restriction digestion is preferred it is nevertheless an optional step.

The present invention further provides a corresponding immunological based differential assay. Accordingly, the present invention provides for antibodies, monoclonal or polyclonal to peptides,
20 polypeptides or proteins encoded by the nucleic acid molecules used in the differential genetic assay.

Yet another aspect of the present invention contemplates a method for detecting *C. granulomatis* in a sample said method comprising subjecting said sample to PCR using a set of nucleic acid
25 probes or primers which are capable of amplifying *C. granulomatis phoE* and incorporating a label on one end of the PCR product and a capture sequence on the other end of the PCR product, subjecting said PCR product to *HaeIII* digestion before or after immobilisation and then detecting the presence of the label wherein the presence of label is indicative of *C. granulomatis*.

30 Preferably, the label is biotin, a fluorophore, an enzyme or a chemical isotope.

Most preferably, the label is biotin.

Preferably, the primer is *phoEdig2*.

5 The present invention further contemplates a range of kits and diagnostic reagents for detecting *C. granulomatis* and, consequently, donovanosis. For example, a kit may be in compartmental form comprising reagents for use in, for example, PCR, oligonucleotide hybridization, RFLP, SSCP, nucleotide sequencing or restriction digestion. The compartments would also contain . . nucleic acid probes such as *Klebsiella phoE* or *Klebsiella scrA* amongst a range of other
10 candidates.

The kit may also be in the form of diagnostic applicator. For example, the applicator may have a nucleic acid probe immobilised thereon. The applicator and immobilised probe is brought into contact with a lesion or ulcer and then placed in suitable reagents for PCR or other suitable
15 nucleic acid detection protocols. The reagents may also be impregnated in the applicator and a positive result may be in the form, for example, of a colour change.

The present invention further contemplates a method of treatment involving antisense nucleotides derived from *Klebsiella* species.

20

The present invention is further described by the following non-limiting Examples.

25

EXAMPLE 1

SAMPLES

Specimens were obtained from patients with lesions consistent with a clinical diagnosis of
5 donovanosis who attended remote community health centres or urban medical services over a
twelve month period. At least two punch biopsies or swabs were obtained from the lesions after
washing the area with saline. An impression smear using a glass slide and swabs for microscopy
and routine culture were also taken. One punch biopsy was processed for histopathology.
10 Tissue sections were stained in 10% w/v giemsa overnight (7) and examined for the presence of
typical histopathological features of donovanosis, in particular the presence of Donovan bodies.

The second punch biopsy or swab was processed for PCR as were swabs from patients with
unrelated genital lesions i.e. herpes genitalis, syphilis, hepatitis infection. Specimens were coded
and laboratory personnel were blinded to the clinical diagnosis.

15

Isolates of *K. pneumoniae* (S1650), *K. ozaenae* (I436) and *K. rhinoscleromatis* (NCTC5046)
were obtained from the Royal Darwin Hospital Culture Collection, Darwin, Northern Territory,
Australia, and processed as positive PCR controls.

20

EXAMPLE 2

DNA EXTRACTION

a) **Punch biopsies:** Punch biopsy specimens were ground in saline with a sterile tissue grinder.
Homogenised specimens were subjected to proteinase K digestion and salt/chloroform extraction
25 (8). Extraction mixes were vortexed vigorously for 5 minutes.

b) **Swabs:** Swab samples were soaked in saline and vortexed for 1-2 minutes. After
centrifugation (12,000 x g for 10 minutes) the pellet was resuspended in 100µl 50mM
NaOH (9).

30

Each tube was overlayed with paraffin oil and incubated at 95°C for at least 15 minutes.

Samples were then neutralised with 16µl 1M Tris-HCl pH8.0 and stored at 4°C until required for PCR.

c) *Klebsiella*: *Klebsiella* isolates were grown on agar plates. One to ten colonies of each isolate were resuspended in 50mM NaOH and treated in the same manner as above.

EXAMPLE 3 PRIMERS

10 a) **Sucrose regulon**: Multiple primers were designed to target the *Klebsiella* sucrose regulon (Genbank accession numbers X61004 and X57401). Figure 1 shows the arrangement of the sucrose regulon in *Klebsiella* and the relative positioning of primers used in this study. Primer sequences are as follows:

15 F1 5' AAAATCTGGGTACTCGGCGA 3' [SEQ ID NO:1]
F2 5' CTGGTGAACCTATACCTAAGC 3' [SEQ ID NO:2]
F3 5' TGGATGTGAACTATATGCGC 3' [SEQ ID NO:3]
F4 5' TGCTGTCCAACATCTTCGTG 3' [SEQ ID NO:4]
F5 5' TCCTGACGGTGATTATCTCC 3' [SEQ ID NO:5]
20 F6 5' TGCTGAACGATCCTAACGGC 3' [SEQ ID NO:6]
R1 5' GATATTGGGGTCGAAGCTGA 3' [SEQ ID NO:7]
R2 5' GACCATAAAGGTAAAGGTGC 3' [SEQ ID NO:8]
R3 5' GTAAACATGTCCGGTAACGCG 3' [SEQ ID NO:9]
R4 5' ATAATCACCGTCAGGAACGG 3' [SEQ ID NO:10]
25 R5 5' AAGCCGTTAGGATCGTTCAG 3' [SEQ ID NO:11]
R6 5' GCACTCCCACATGTAGCCG 3' [SEQ ID NO:12]

Thirteen different primer combinations were tested in the PCR. These were F1/F1; F2/R1; F2/R3; F3/R1; F3/R2; F3/R3; F4/R4; F4/R5; F4/R6; F5/R5; F5/R6; F6/R5 and F6/R6.

30

b) *phoE/scrA* PCR evaluation: Two sets of primers (A & B) were selected for use in the PCR

evaluation. These two primers sets are among the most sensitive, robust and *Klebsiella* specific primer sets of the many tested.

Primer set A was designed to amplify a 382bp region of the *Klebsiella phoE* gene (5' CTATGACAGCAAGGATGGCGA3' [SEQ ID NO:13]) and (5'CTGGTACTGCAGGGTCAGA 3' [SEQ ID NO:14]) whilst primer set B (previously designated F4/R4) was designed to amplify a 438bp region of the *Klebsiella scrA* gene [Genbank accession number X57401] (5' TGCTGTCCAACATCTTCGTG 3'[SEQ ID NO:4]) and (5' ATAATCACCGTCAGGAACGG 3' [SEQ ID NO:10]).

10

c) PCR for sequencing: Primer set *phoEsseq* [5' ACCTACCGCAACACCGACTTCTTCGG 3' (SEQ ID NO:15)] and 5' TGATCAGAACTGGTAGGTCAT 3' (SEQ ID NO:16)] was designed to amplify a 604 bp product of the *Klebsiella phoE* gene. This same primer set was used for the sequencing of amplified products.

15

d) PCR for restriction digests: Primer set *phoEdig1* [5' CTATGACACGAAGGATGGCGA 3' (SEQ ID NO: 17) and 5' CAGACCGAAGTCGAACTGATACTG 3' (SEQ ID NO: 18)] was designed to amplify a 700bp region of the *Klebsiella phoE* gene whilst primer set *phoEdig2* [2' GATCTGACCCTGCAGTACC 3' (SEQ ID NO: 19) and 5' TCCGCTTTCGAACCCTGG 3' 20 (SEQ ID NO: 20)] was designed to amplify a 188bp region of the *Klebsiella phoE* gene.

EXAMPLE 4

POLYMERASE CHAIN REACTION

25 The final reaction mixture contained 1 μ l of template DNA, 0.5mM primers, 0.2 μ M deoxynucleotide triphosphates, 1 x PCR buffer (10mM Tris-HCl pH 8.0, 50mM KCl, 2.5mM MgCl₂, 0.01% w/v gelatin) and 0.5U of Taq polymerase in a final volume of 25 μ l. Samples were subjected to 30 cycles of denaturation (94°C, 40 seconds), annealing (55°C, 40 seconds) and extension (72°C, 60 seconds) in a thermal cycler (Corbett Research, Sydney, Australia).

30

Positive and negative controls for the DNA extraction and PCR procedures were included. PCR

products were analysed by 1% w/v agarose gel electrophoresis.

EXAMPLE 5

SEQUENCE ANALYSIS

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Samples which tested positive for *C. granulomatis* with the *phoE/scrA* PCR (primer sets A and B) were reamplified with the *phoEseq* primer set (604bp). Products of the expected size were purified and sequenced in both directions using the dye terminator method (Applied Biosystems).

.. Templates that were difficult to sequence via the automated method were blunt end cloned (10)
10 into pBluescript (SK-, Pharmacia - Biotech) and manually sequenced in both directions using a T7 sequencing kit (Pharmacia-Biotech).

EXAMPLE 6

*Hae*III RESTRICTION DIGEST #1

15

Samples for which sequencing was carried out and samples of non-sequenced positive PCR samples were subjected to amplification with the *phoEdig1* primer set (700bp). Isolates of *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis* were also amplified as was the single sample that gave a indeterminate result in the *phoE/scrA* PCR. Amplified products were digested with
20 *Hae*III for 2 hours at 37°C in the following digest mix: 1 x OPA (One-Phor-All buffer PLUS, Pharmacia-Biotech), 10-15 µl amplified PCR product, 2U *Hae*III (Pharmacia-Biotech) and dH₂O to a final volume of 20 µl. After incubation at 37°C the digestion mixes were incubated at 85°C for 30 minutes in order to inactivate the enzyme. Digested products were analysed by 2% w/v low melting point agarose electrophoresis (NUSIEVE, FMC BioProducts), designed for the
25 separation of low molecular weight fragments.

EXAMPLE 7

***HaeIII* RESTRICTION DIGEST #2**

Of the samples that tested positive for *C. granulomatis* with the original *phoE/scrA* PCR, a number which were negative samples or indeterminate samples which originally tested positive using *phoE/scrA* PCR contained negative samples and indeterminate samples. These were then tested with the *phoEdig2* primer set (188bp) along with the aforementioned *Klebsiella* isolates in order to simplify the post-digest fragment mixture. The procedure and conditions for restriction digest were simplified by the addition of 2U *HaeIII* directly to the post-PCR mix. Incubation temperatures, times and analysis of digested fragments were identical to those outlined for *HaeIII* restriction digest #1.

EXAMPLE 8

DETECTION OF *C. granulomatis*

15

a) Sucrose regulon: PCR products were obtained with *Klebsiella* DNA for 11/13 primer combinations. Failed combinations, F3/R3 and F6/R5, were discarded from further use. For the remaining 11 combinations, products of the expected size were consistently obtained with DNA prepared from *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis*, but no products have ever been obtained with *C. granulomatis* DNA.

These results suggested that *C. granulomatis* lacks the *Klebsiella* sucrose regulon, enabling the inventors to discriminate between these closely related organisms at the molecular level.

25 b) Clinical PCR evaluation

i) Routine Culture: Morphologically, donovan bodies are indistinguishable from free growing *Klebsiella*. Swabs from each patient were, therefore, subjected to routine culture. The swab specimen that gave products for both *phoE* and *scrA* was the same specimen that grew *Klebsiella* in routine culture.

ii) **Histopathology:** Most of the specimens obtained from patients with lesions clinically consistent with a diagnosis of donovanosis were considered adequate for histological studies. Histological detection of the typical Donovan bodies were possible approximately half the cases and the remaining cases were reported as negative for Donovan bodies.

5

iii) **Polymerase Chain Reaction:** All of the above specimens and the specimens from unrelated genital lesions were considered adequate for PCR with *phoE* and *scrA* primer sets. None of the specimens from the latter group gave PCR products. Of the clinical donovanosis specimens, most were positive for the *phoE* product and negative for the *scrA* product.

10

Figure 2 shows representative *phoE/scrA* PCR results for the internal *Klebsiella* PCR controls, for patients with clinically diagnosed donovanosis and for patients with unrelated genital lesions. A 2 x 2 analysis (Table 1) shows that the sensitivity of this PCR is 100% and the specificity 29% when compared to the gold standard of histopathology. Alternatively, when the comparison is made with the clinical diagnosis the sensitivity of the PCR is shown to be 87% and the specificity 100%. These results indicate that this PCR is more sensitive than the histopathological method for the diagnosis of donovanosis.

A number of the PCR specimens received were swabs. After discarding the specimen which grew *Klebsiella* in routine culture, all swab specimens confirmed the clinical diagnosis of donovanosis compared to approximately 70-80% of biopsy specimens. This indicates that swabs are at least as sensitive as biopsies in the diagnostic PCR.

The inventors have demonstrated that it is possible to use PCR to diagnose donovanosis and this represents an important addition to the diagnostic modalities available to aid in the management of this often neglected and poorly understood condition.

Determining the sensitivity and specificity of PCR diagnostics is problematic as the method is potentially more sensitive than the accepted gold standards, against which the new technology is measured. It is well known that histology is positive in only 60-80% of cases where donovanosis is the clinical diagnosis and other causes of ulceration have been excluded. The

PCR is 100% sensitive when compared to histology and returned a positive result in cases of clinically diagnosed donovanosis which were histologically negative. The PCR assay was negative on all negative clinical control specimens.

5

EXAMPLE 9

EVALUATION OF THE DIAGNOSTIC POTENTIAL OF THE LACK OF SUCROSE REGULON IN *C. granulomatis*

.. A surprising finding in Example 8 was that primers F1 and R1 (Figure 1), corresponding to *scrK*
10 of the *Klebsiella* sucrose regulon did not yield any product with *C. granulomatis* template. Lack of the *scrK* product may be due either to the lack of the *scrK* gene or divergence of its sequence. With a view to distinguishing between these two possibilities primer-sets when designed corresponding to other genes within the sucrose regulon (Figure 1).

15 Of 13 combinations tested, again 11 gave expected products with *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis* templates. On the other hand, one of the latter 11 primer-sets gave amplification products derived from *C. granulomatis* template (Figure 3). It is proposed, therefore, that the sucrose regulon is lacking in *C. granulomatis*. The lack of a sucrose regulon could potentially be a diagnostic character if DNA from all independent specimens exhibits the
20 same property.

To further test the diagnostic potential of this observation, PCR was performed with the *phoE* and *scrA* specific primers (primers sets A and B). Template DNA was extracted from 34 specimens from patients with lesions clinically suggestive of donovanosis for which comparative
25 histology was also available. Of the 34 specimens, 11 (38%) returned a positive histology result for donovanosis whilst 20 (59%) gave the expected PCR profile with the *phoE/scrA* primer set (Figure 4). No samples for which a positive histology result was confirmed were negative by PCR. One specimen gave an indeterminate PCR result with products being obtained for both *phoE* and *scrA*. The histological diagnosis for this specimen was Vaginal Intraepithelial
30 Neoplasia suggesting that the inventors had either amplified a contaminating *Klebsiella* or that some other unidentified organism was cross reacting with the primer set. Interestingly,

specimens had been received from the same patient twice previously and had tested PCR negative for donovanosis on both occasions. Both sets of primers gave products of expected size with *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis*. DNA from specimens from patients with unrelated genital conditions (herpes genitalis, syphilis, gonorrhoea, human papillomavirus infection, mixed anaerobic infection) did not give products with either set of primers (Figure 4).

No significant difference was observed between the performance of swab samples and biopsy samples in PCR analysis with the ratio of positive biopsy PCR : positive histology being 10:5 and the ratio of positive swab PCR : positive histology being 12:7. PCR positive patients had both swab and biopsy specimens taken. Results were concordant and have been included in the preceding ratios.

In order to confirm that the *phoE* products amplified were actually derived from *C. granulomatis*, the positive PCR specimens were amplified with primer set *phoEseq* (604bp) and sequenced. The sequence results for all nine samples showed then Arg → Cys change for *C. granulomatis*.

EXAMPLE 10

DIFFERENTIATION OF *C. granulomatis* FROM THE KLEBSIELLA BY RESTRICTION DIGEST OF *phoE* PRODUCTS

Analysis of the *phoE* sequence from *C. granulomatis*, *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis* showed that there were a total of two base changes that were unique to *C. granulomatis*. These changes corresponded to position 502 of the coding nucleotide sequence (C→T) which resulted in Arg → Cys change and position 584 of the coding nucleotide sequence (G → A) which resulted in a silent change. Furthermore, it was also noticed that both of these unique changes resulted in the elimination of *HaeIII* restriction sites. Previously sequenced positive PCR samples and the non-sequenced positive PCR samples were, therefore, amplified with the *phoEdig1* primer set and digested with *HaeIII*, along with DNA obtained from isolates of *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis* (Figure 5). The sample for which the indeterminate PCR results were obtained was also amplified and digested. The inventors were

able to distinguish the *C. granulomatis* derived *phoE* product from that of the *Klebsiella* by the presence of a 167bp fragment resulting from the eliminated *HaeIII* sites (Figure 6). The indeterminate sample digested into a single band of ~100 bp in length, suggesting that it was derived neither from contaminating *Klebsiella* nor from *C. granulomatis* with the presence of
5 a sucrose regulon.

The combination of the *phoE/scrA* PCR diagnostic with *HaeIII* restriction digest, therefore, has advantages over the PCR diagnostic alone. In particular, if contaminating *Klebsiella* species (or other unidentified cross-reacting organisms) are masking infection with *C. granulomatis* in the
10 *phoE/scrA* PCR, *C. granulomatis* should be uncovered in the *HaeIII* restriction digest. This assumes that both *C. granulomatis* and *Klebsiella* products have been amplified in the one visible band. Furthermore, the validation of positive PCR results by restriction digest is a much faster process than sequence analysis, whilst giving the same essential information with regards to the two unique base changes.

15

By targeting the 167bp differential fragment of *C. granulomatis*, the inventors were able to considerably simplify the patterns to be distinguished. Samples positive for *C. granulomatis* with the *phoE/scrA* primer sets were tested with the *phoEdig2* primer set. DNA from isolates of the aforementioned *Klebsiella* and from the indeterminate PCR sample were also amplified, as were
20 all negative samples and the samples from patients with unrelated genital conditions. All samples positive for *C. granulomatis* with the *phoE/scrA* primer sets were also positive with the *phoEdig2* primer set, as were the *Klebsiella* and the only indeterminate sample. All samples from patients with unrelated genital lesions were negative, as were the original comparative negative samples. Comparative samples for which a negative result was obtained with the
25 *phoE/scrA* primer sets gave a very weak positive result with the *phoEdig2* primer set. Interestingly, the final clinical diagnosis for these patients remained donovanosis, with one patient also diagnosed with carcinoma.

The samples positive for the 188bp *phoEdig2* PCR fragment were digested with *HaeIII*, along
30 with products for the *Klebsiella* and indeterminate sample. Band sizes expected from the sequence data were obtained for all positive samples and were once again distinguishable from

the *Klebsiella* and indeterminate sample.

A modification of the PCR-*Hae*III digest is proposed so that it may articulate with EIA based PCR diagnostic systems. Importantly, the NaOH DNA preparation method that the inventors have employed is compatible with this system. In *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis*, the cleavage of the fragment containing the capture oligo target region from the biotinylated fragment will give a negative result in the EIA based assay whilst the intact fragment of *C. granulomatis* will give a positive result.

10

EXAMPLE 11

ASSAY FOR *C. granulomatis*

Figure 7 shows a convenient assay for *C. granulomatis* based on the PCR/*Hae*III procedure. The 188bp *phoEdig2* PCR product is subjected to *Hae*III digestion. Magnetic beads carrying capture oligonucleotides are then optionally added to immobilise the PCR product. In the absence of *Hae*III digestion, the *phoEdig2* PCR product remains intact and the biotin label is detected indicating the presence of *C. granulomatis*. If the biotin label is not detected, *Hae*III cleavage has occurred and the diagnosis is the absence of *C. granulomatis*.

20 In a modification to this assay, a protein binding DNA sequence (e.g. GCN4) is incorporated into one end of the *phoEdig2* PCR product using an appropriate primer. This PCR product is then immobilised using the capture sequence and subjected to *Hae*III digestion. After suitable washing steps, the presence of biotin is indicative of *C. granulomatis*.

25 In a further alternative, prior to immobilisation, the product is amplified with and without *Hae*III. Where a positive biotin signal is given in both instances, this indicates the presence of *C. granulomatis*. If a positive signal is given without *Hae*III but a negative result is given with *Hae*III, then this indicates the absence of *C. granulomatis*.

30 Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that

the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: AMRAD OPERATIONS PTY LTD

(ii) TITLE OF INVENTION: A METHOD OF DIAGNOSIS AND AGENTS USEFUL
FOR SAME

(iii) NUMBER OF SEQUENCES: 18

(iv) CORRESPONDENCE ADDRESS:

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(D) STATE: VICTORIA

(E) COUNTRY: AUSTRALIA

(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAAATCTGGG TACTCGGCGA

20

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTGGTGAAC ATACCTAAGC

20

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGGATGTGAA CTATATGCGC

20

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGCTGTCCAA CATCTTCGTG

20

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCCTGACGGT GATTATCTCC

20

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGCTGAACGA TCCTAACGGC

20

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATATTGGGG TCGAAGCTGA

20

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GACCATAAAG GTAAAGGTGC

20

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTAAACATGT CCGTAACGCG

20

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATAATCACCG TCAGGAACGG

20

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AAGCCGTTAG GATCGTTCAG

20

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCACTCCAC ATGTAGCCG

19

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTATGACAGC AAGGATGGCG A

21

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTGGTACTGC AGGGTCAGA

19

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTATGACACG AAGGATGGCG A

21

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CAGACCGAAG TCGAACTGAT ACTG

24

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GATCTGACCC TGCAGTACC

19

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TCCGCTTTTCG AACCCCTGG

18

TABLE 1
A COMPARISON OF DIAGNOSTIC METHODS FOR DONOVANOSIS

		HISTOPATHOLOGICAL DIAGNOSIS		CLINICAL DIAGNOSIS	
		+	-	+	-
PCR DIAGNOSIS	+	8	5	13	0
	-	0	2	2	8*

*not examined histologically

CLAIMS:

1. A method of detecting *Calymmatobacterium* species in a sample said method comprising detecting the presence or absence of a nucleotide sequence of said *Calymmatobacterium* species or an organism related thereto wherein the presence or absence of said nucleotide sequence is indicative of *Calymmatobacterium* species in the sample.
2. A method according to claim 1 wherein the *Calymmatobacterium* species is *Calymmatobacterium granulomatis*.
3. A method according to claim 2 wherein the sample is a lesion or ulcer from the genital region or surrounding area.
4. A method according to any one of claims 1 to 3 wherein the method comprises using a nucleic acid probe and/or primer directed to a genetic sequence substantially absent in *C. granulomatis* unique from *C. granulomatis*.
5. A method according to any one of claims 1 to 4 wherein the detection of presence or absence of a nucleotide sequence is accomplished using a nucleic acid probe and/or primer directed to a genetic sequence from a genetically related organism.
6. A method according to claim 5 wherein a genetically related organism is *Klebsiella* species.
7. A method according to claim 6 wherein the *Klebsiella* species is selected from *Klebsiella pneumoniae*, *Klebsiella ogaenae* and *Klebsiella rhinoscleromatis*.
8. A method according to claim 5 wherein the nucleic acid probe and/or primer is directed to the genetic sequence encoding phosphate porin (*phoE*), its ribosomal RNA, glutamate dehydrogenase (*gdhA*), acetolactate synthase (*iluk*) or a gene from the sucrose regulon (*scr*).
9. A method according to claim 4 wherein the genetic sequence absent from *C.*



granulomatis is a *scr* gene.

10. A method according to claim 8 wherein the *scr* gene is *scrA*.

11. A method of detecting *C. granulomatis* in a sample said method comprising detecting the presence of a first nucleotide sequence with a nucleic acid molecule from an organism other than *C. granulomatis* wherein first nucleotide sequence is substantially represented in both said organism and *C. granulomatis* and then determining the presence or absence of a second nucleotide sequence which is absent from *C. granulomatis* but present in said organism using another nucleic acid molecule, wherein the presence of said first nucleotide sequence and absence of said second nucleotide sequence is indicative of *C. granulomatis* in said sample.

12. A method according to claim 11 wherein the first nucleotide sequence detected is related to *Klebsiella phoE*.

13. A method according to claim 12 wherein the second nucleotide sequence detected is related to *Klebsiella scrA*.

14. A method of detecting *C. granulomatis* in a sample, said method comprising isolating genetic sequences having a high degree of nucleotide similarity from *C. granulomatis* and a species of *Klebsiella*, digesting said genetic sequences with a restriction endonuclease which cleaves the genetic sequence from *C. granulomatis* resulting in a restriction pattern different to the pattern obtained when the same enzyme cleaves the genetic sequence from a *Klebsiella* species wherein the presence of a particular restriction endonuclease profile is indicative of whether the sample contains *C. granulomatis* or a *Klebsiella* species or neither of organism.

15. A method according to claim 14 wherein the genetic sequences having a high degree of similarity from *C. granulomatis* and *Klebsiella* sp is *phoE*.

16. A method according to claim 15 wherein the restriction endonuclease is *HaeIII*.





17. A method for diagnosing donovanosis, said method comprising collecting a sample from a lesion and determining the presence or absence of a genetic sequence in *C. granulomatis* wherein the presence or absence of such a sequence is indicative of donovanosis.

18. A method according to claim 17 wherein the method comprises determining the absence of *scrA*.

19. A method according to claim 17 or 18 wherein the method comprises determining the presence of a *phoE* gene having a *HaeIII* restriction pattern which is different to *HaeIII* restriction pattern from *Klebsiella phoE* gene.

20. A method for detecting *C. granulomatis* in a sample said method comprising subjecting said sample to PCR using a set of nucleic acid probes or primers which are capable of amplifying *C. granulomatis phoE* and incorporating a label on one end of the PCR product and a capture sequence on the other end of the PCR product, subjecting said PCR product to *HaeIII* digestion before or after immobilisation and then detecting the presence of the label wherein the presence of label is indicative of *C. granulomatis*.

21. A kit comprising in compartmental form reagents required for performing the method according to any one of claims 1 to 20.

22. A method according to any one of claims 1 to 20 or a kit according to claim 21 substantially as hereinbefore described with reference to the Figures and/or Examples.

