

Original
articleA colorimetric detection system for
Calymmatobacterium granulomatis

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Objective: To incorporate the first polymerase chain reaction (PCR) assay for *Calymmatobacterium granulomatis* into a colorimetric detection system for use in routine diagnostic laboratories.

Methods: A capture oligonucleotide specific for the *Klebsiella phoE* gene was covalently linked to tosyl activated magnetic beads. Biotinylated *phoE* PCR products obtained from 14 positive specimens from patients with donovanosis and isolates of *Klebsiella pneumoniae*, *K rhinoscleromatis*, and *K ozaenae* were cleaved with *HaeIII* for the purpose of differentiation, captured by the prepared beads, and subjected to standard EIA detection methodology. Eight samples from unrelated genital conditions underwent the same procedure. It was anticipated from the sequence data that the biotinylated fragment would be cleaved from the capture oligonucleotide target region in the three *Klebsiella phoE* products (that is, a negative colorimetric result) while the entire fragment of interest would remain intact in the positive *C granulomatis phoE* products (that is, a positive colorimetric result).

Results: All 14 positive specimens from patients with donovanosis gave strong colorimetric readings with this detection system. Isolates of *K pneumoniae*, *K rhinoscleromatis*, *K ozaenae*, and the eight specimens from unrelated genital conditions were negative.

Conclusion: The successful development of a colorimetric detection system for *C granulomatis* incorporating two levels of specificity enables the molecular diagnosis of this condition to be undertaken by routine diagnostic laboratories. This should have an important role in the Australian government's campaign to eradicate donovanosis by 2003 though the test still needs to undergo trials and be validated using a larger number of samples from geographically diverse parts of the world in order to ascertain the generalisability of the methodology.

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Keywords: donovanosis; *Calymmatobacterium granulomatis*; molecular diagnosis; colorimetric detection

Donovanosis is a chronic genital ulcerative disease with endemic foci in socially disadvantaged populations throughout the world.¹ In recent years there has been a resurgence of interest in this disease owing to the association of genitoulcerative disease with an increased risk of HIV-1 transmission and acquisition. While unproved, there is a highly likely synergistic link between HIV-1 and donovanosis.² The Australian government has a target to eradicate donovanosis by 2003.³

The causative organism of donovanosis, *Calymmatobacterium granulomatis*, has been associated with the genus *Klebsiella* on the basis of morphological, ultrastructural, and antigenic studies.^{4,5} Furthermore, we have recently reported phylogenetic evidence for its reclassification as *Klebsiella granulomatis* comb nov.⁶

Previously we reported the development of the first polymerase chain reaction (PCR) assay for the detection of *C granulomatis* in clinical specimens.⁷ The specificity of the PCR assay is based on two unique base changes in the *phoE* gene of *C granulomatis* which eliminate *HaeIII* restriction sites, enabling clear differentiation from closely related species of *Klebsiella*. A major advantage of this test is that it can be utilised with swab rather than biopsy/tissue smear specimens. It was suggested in this report that it should now be possible to incorporate this PCR/*HaeIII* digest into a colorimetric detection system for use in routine diagnostic laboratories. We now report a colorimetric

detection system for the detection of *C granulomatis* from clinical specimens. There is no intent to present this work as a validation trial. Rather, our intent is to make this methodology available to as many researchers/medical practitioners as possible so that appropriate validation trials may be carried out.

DNA extractions and amplification of 188 bp *phoE* products were carried out as previously reported,⁷ with the exception that a biotin group was attached to the 5' PCR primer. The PCR products were then cleaved by addition of 1 unit of *HaeIII* and incubation at 37°C for 1 hour. Tosyl activated magnetic beads were obtained (Dynal, Carlton, Victoria, Australia) and washed as recommended by the manufacturer. A capture oligonucleotide with 5' amine group was designed internal to the *HaeIII* restriction sites of the *C granulomatis phoE* product (5'NH₂CCAGCAGGTTCTGATC GTT3') and added to the beads at 150 pmol/mg beads in 0.1 M borate buffer pH 9.5. Incubation at 65°C for 15 minutes allowed covalent bond formation between the tosyl groups and 5' amine groups. Free tosyl groups were blocked with 0.2 M ethanolamine pH 7.0 containing 0.1% bovine serum albumin for 4 hours at 37°C. Before use the beads were diluted 1 in 4 with Probe Suspension 2, pH 4.0 (sodium phosphate solution containing <0.2% solubiliser and <26% chaotrope, contained within Cobas Amplicor *Chlamydia trachomatis* Detection Kit, Roche Diagnostic Systems, Inc,

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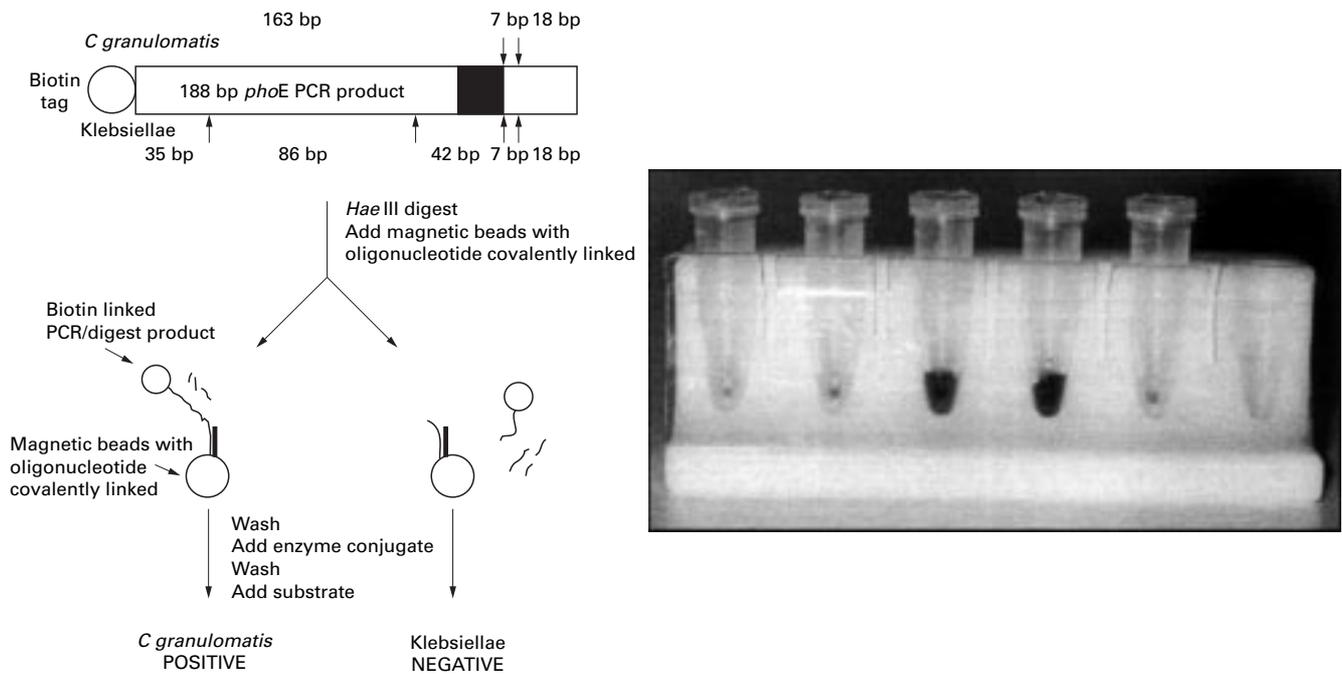


Figure 1 Left: Schematic representation of the colorimetric detection system for *C. granulomatis*. Arrows represent *Hae*III restriction sites within the 188 bp *phoE* product for *C. granulomatis* (above) and other *klebsiellae* (below). The black box within the 188 bp product represents the target region for the capture oligonucleotide. Right: Results from left to right: *K. pneumoniae*; *K. rhinoscleromatis*; positive swab sample from patient with donovanosis; positive swab sample from patient with donovanosis; *K. ozaenae*.

Branchburg, NJ, USA). PCR/*Hae*III digest products (25 μ l) were denatured with 0.1 M sodium hydroxide (25 μ l). The denatured solution (30 μ l) was incubated with 100 μ l of prepared beads for 30 minutes at 37°C to allow for neutralisation and binding with the capture oligonucleotide. All subsequent steps of detection were carried out using the standard EIA methodology (that is, wash, add avidin-HRP conjugate, wash, add substrate). A magnetic particle concentrator (Dyna, Carlton, Victoria, Australia) was utilised at each step to separate the beads from the supernatant and allowed all steps to be carried out in one tube.

A schematic representation of this system is provided (fig 1).

The sample set tested with this detection system was identical to that previously reported,⁷ and included 14 positive samples from patients with donovanosis, eight samples from patients with unrelated genital conditions (herpes genitalis, syphilis, gonorrhoea, human papillomavirus infection, and mixed anaerobic infection—there is no reported chancroid or lymphogranuloma venereum in the Northern Territory of Australia), and isolates of *K. pneumoniae*, *K. ozaenae*, and *K. rhinoscleromatis*. Owing to the cleavage of the fragment containing the biotin group from the capture oligonucleotide target region it was expected that the three *Klebsiella* digest products would give negative readings while the intact *C. granulomatis* fragment would give positive readings.

Strong colorimetric readings were obtained for all 14 positive samples from patients with donovanosis. All eight samples from patients with unrelated genital conditions were negative as were PCR digests obtained from the three *Klebsiella* isolates (fig 1).

This methodology has advantages over agarose gel electrophoresis in that it can be utilised

by routine diagnostic laboratories and incorporates a second level of specificity via the presence of an internal capture oligonucleotide.

All 14 positive samples from patients with donovanosis included in this report were obtained from the Top End of the Northern Territory of Australia over a 2 year period. However, the test has been/is being successfully utilised by doctors in Central Australia, South Australia, and Western Australia (data not shown as ethical clearance applies only to Top End of the Northern Territory) and at the very least should be particularly valuable if there is a serious attempt to eradicate donovanosis in Australia.

It is interesting to note that the base change at position 532 of the coding nucleotide sequence (C→T) results in the amino acid change Arg157→Cys157. Arg157 is located near the top of one of the surface exposed loops in the *Klebsiella PhoE* protein and corresponds to Arg158 of the *Escherichia coli PhoE* protein.⁸ Korteland *et al* reported that all *E. coli* K 12 strains tested in their laboratory with resistance to the *PhoE* specific bacteriophage TC45 showed a mutation at Arg158.⁹ While unproved, it is plausible that the *C. granulomatis* isolates present in Australia have also acquired this selective advantage at some point in their evolution.

This gives some strength to the future stability of this mutation in the Australian context though the question still remains as to whether the organism is clonal in an international context. The specificity of the PCR assay still needs to undergo trials and be validated using a larger number of samples from geographically diverse locations within and outside Australia in order to test the stability and applica-

bility of the two unique base changes on which it relies.

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Joint Meeting of MSSVD and the ASTDA

3–7 May 2000, Baltimore Marriott Inner Harbor Hotel, Baltimore, Maryland, USA

To mark the unique nature of the millennial year, for the first time in its 78 year history the Medical Society for the Study of Venereal Diseases will hold its Spring Meeting jointly with the American Sexually Transmitted Disease Association (President, Professor Julius Schacter). Our local host will be Professor Jonathan Zenilman of Johns Hopkins Medical School. The scientific programme will consist of plenary lectures and round table discussions, delivered by world authorities. There will also be oral and poster presentations of original work.

Further mailings will follow to MSSVD and ASTDA members. People who do not belong to either of these organisations and who would like to receive further information should contact: Dr Keith Radcliffe, Honorary Assistant Secretary, MSSVD (fax: +44 (0) 121-237 5729; email: k.w.radcliffe@bham.ac.uk).