

Laboratory
Medicine

Non-cultural detection of rectal and pharyngeal gonorrhoea by the Gen-Probe® PACE® 2 Assay

H Young, J Anderson, A Moyes, A McMillan

Objective: To assess the sensitivity and specificity of the Gen-Probe® PACE® 2 assay, which uses a chemiluminescent labelled single-stranded DNA probe to detect gonococcal ribosomal RNA (rRNA), for the non-cultural detection of rectal and pharyngeal gonorrhoea in homosexual men.

Subjects: 161 homosexual men attending the Department of Genitourinary Medicine, Edinburgh Royal Infirmary during the latter half of 1995 and the first quarter of 1996.

Methods: Duplicate rectal and pharyngeal swabs were collected for culture on modified New York City (MNYC) medium and detection of gonococcal nucleic acid by the Gen-Probe assay. Repeatedly reactive Gen-Probe specimens from culture negative patients were also tested by the Gen-Probe competition assay (PCA).

Results: Of the 161 patients, 23 (14.3%) gave a positive culture at one or both sites (rectum 10, throat 8, rectum and throat 5) compared with 28 (16.7%) who gave a positive Gen-Probe result at one or both sites (rectum 9, throat 11, rectum and throat 8). After resolution of discrepant results by PCA the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Gen-Probe was 94.1%, 100%, 100% and 99.3% for rectal specimens while the corresponding values for pharyngeal specimens were 86.4%, 100%, 100%, and 97.9%. The sensitivity and NPV of rectal culture were 88.2% and 98.6% while the corresponding values for pharyngeal culture were 59% and 93.9%. Gen-Probe was significantly more sensitive than throat culture ($p < 0.05$) but not rectal culture ($p > 0.2$). The average Relative Light Units (RLU) value for the cut-off was 386 (range 351-450) while the average for a positive result was 20306 (range 403-110 104): this was, however, significantly higher ($p = 0.019$) in rectal specimens 31325 (range 1705-110 104) than in throat specimens 10 447 (range 403-15 633).

Conclusions: Gen-Probe® PACE® 2 assay is a sensitive and specific method for the detection of rectal and pharyngeal gonorrhoea. As the Gen-Probe assay may detect nucleic acid from non viable gonococci the clinical significance of a probe positive culture negative specimen from a patient without culture evidence of gonorrhoea at another site is uncertain and requires further consideration. Nevertheless a positive result does indicate exposure to infection and could be important in ensuring appropriate partner notification action. If non-cultural methods are used to screen for gonococcal infection cultures should be obtained from patients with positive results in order that the antibiotic susceptibility and molecular epidemiology of the gonococcal population can be monitored.

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Keywords: *Neisseria gonorrhoeae*; DNA probe; pharynx; rectum

Introduction

The epidemiology of gonorrhoea has changed in recent years with a much greater proportion of infections now acquired by homosexual contact.^{1,2} Rectal and pharyngeal gonorrhoea has also increased in importance as infection at these sites is more common in homosexual men. Rectal gonorrhoea is recognised as an independent risk factor for HIV infection in homosexual men.^{3,4} The prevalence of rectal gonorrhoea in men has also been suggested as a rapid method of estimating the amount of unsafe sexual behaviour⁵ and as such can be used to assess the effectiveness of health education and prevention programmes in homosexual men. As HIV infection may also be acquired through oro-genital sex^{6,7} the high levels of pharyngeal gonorrhoea, over 50% recorded in homosexual men in Newcastle¹ and Edinburgh⁸ are a cause for concern.

Difficulties in the cultural diagnosis of rectal and pharyngeal gonorrhoea include issues of sensitivity, related to low numbers of organ-

isms, and specificity, due to the occurrence of non-gonococcal neisseriae such as *N meningitidis* which are frequently isolated from the throat and occasionally from the rectum.⁹ Non-cultural methods of detecting gonococcal infection should overcome culture-associated problems. The Gen-Probe® PACE® 2 assay uses a chemiluminescent labelled single-stranded DNA probe that is complementary to the ribosomal RNA (rRNA) of the gonococcus: rRNA released from gonococci in the specimen combines with the labelled probe to form a stable DNA:RNA hybrid which is measured objectively in a luminometer. Gen-Probe has been extensively evaluated using genital specimens and, in general, high sensitivity has been demonstrated with values of 97.1%,¹⁰ 99.4%,¹¹ and 100%¹² although one recent study reported a sensitivity of 85.9% for endocervical specimens and 91.5% for male urethral specimens.¹³ A preliminary evaluation of Gen-Probe gave a sensitivity of 80% for pharyngeal and 100% for rectal specimens¹⁴

Scottish *Neisseria gonorrhoeae* Reference Laboratory, Edinburgh University Medical School

H Young
J Anderson
A Moyes

Genito-Urinary Medicine Unit, Department of Medicine Edinburgh Royal Infirmary
A McMillan

Address correspondence to:
Dr H Young, Scottish *Neisseria gonorrhoeae* Reference Laboratory, Edinburgh University Medical School, Teviot Place, Edinburgh.

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but it was concluded that further studies were needed.

The objective of this study was to assess the sensitivity and specificity of the Gen-Probe® PACE® 2 assay for the non-cultural detection of rectal and pharyngeal gonorrhoea in homosexual men.

Patients and methods

Specimens and culture procedures

Duplicate rectal and pharyngeal swabs were collected from 161 homosexual men attending the Genitourinary Medicine Unit, Edinburgh Royal Infirmary during the latter half of 1995 and the first quarter of 1996. One swab from each site was plated directly onto modified New York City (MNYC) medium¹⁵ and the plates held at 37°C in a carbon dioxide enriched atmosphere for up to four hours before transfer to the laboratory. Cultures were examined after 24 and 48 hours incubation and any suspect colonies tested by the oxidase test: oxidase positive Gram negative diplococci were confirmed as *N gonorrhoeae* by immunological (Phadebact Monoclonal GC test positive) and biochemical (rapid carbohydrate utilisation) tests¹⁶: non-gonococcal neisseriae were speciated on the basis of their carbohydrate utilisation pattern. The other specimen was collected using the Gen-Probe® PACE® 2 collection swab and transport tube (Gen-Probe Incorporated, San Diego, California). The order of collecting swabs for culture and Gen-Probe was alternated weekly.

Gen-Probe assays

The Gen-Probe collection swabs were stored at -20°C and tested according to the manufacturer's instructions. Testing was performed over 20 runs each of which included the three negative reference specimens and the positive control supplied with the kit. The assays were read using the Gen-Probe LEADER Luminometer and the results calculated on the basis of the difference between the response in Relative Light Units (RLU) of the specimen and the mean of the negative reference: a specimen is considered Positive if the difference is \geq 300 RLU and negative if the difference is less than 300 RLU.

Repeat testing in the case of discrepant results

After comparing the Gen-Probe and culture results all discrepancies were re-tested by Gen-Probe using the original specimen. Repeatedly reactive Gen-Probe specimens from culture negative patients were also tested by the Gen-

Probe competition assay (PCA).^{14,17} In the PCA assay the sample is tested in duplicate tubes one of which is processed by the standard assay procedure while an excess of probe (identical to the probe used in the standard assay except that it lacks the chemiluminescent label) is added to the second tube. A reduction of \geq 70% in the signal generated in the PCA reaction tube containing unlabelled probe compared with the signal generated in the standard tube with only labelled probe indicates that the specimen contains gonococci and did not give a reaction due to interfering material in the sample. In the case of culture positive Gen-Probe negative specimens, the cultured gonococci were also tested by Gen-Probe.

Statistical analysis

Chi square and two sample *t* tests were performed with the Minitab PC software package.

Results

Of the 161 patients, 23 (14.3%) gave a positive culture at one or both sites (rectum 10, throat 8, rectum and throat 5) compared with 28 (16.7%) who gave a positive Gen-Probe result at one or both sites (rectum 9, throat 11, rectum and throat 8); the number of patients detected by each method is not statistically significant ($\chi^2 = 0.58$; $p > 0.3$). The 23 culture positive patients represented infection with seven different serovar/auxotype combinations: IB2/NR (14); IB2/H (4); IB2/P (1); IB3/NR (1); IB7/NR (1); IB8/NR (1); and IB16/NR (1). Meningococci were isolated from 57 (35.4%) of the throat cultures, *N perflava* from 2 (1.2%), *Moraxella catarrhalis* from 2 (1.2%), and *N lactamica* from 1 (0.6%). One rectal culture yielded *N meningitidis*.

The correlation between culture and Gen-Probe is summarised in the table.

Rectal culture versus Gen-Probe The overall agreement was 97.5% (157/161) with three of the four discrepancies due to negative cultures. One of these patients had had rectal gonorrhoea three weeks earlier and one had urethral gonorrhoea at the time of sampling while there was no known risk factor in the case of the third patient. All three positive Gen-Probe results were repeatedly reactive and gave 94%–97% inhibition in the PCA assay confirming that the specimens contained gonococcal nucleic acid. The Gen-Probe negative (RLU 120) culture positive specimen remained negative (RLU 113) on repeat although a positive probe result was obtained when the cultured isolate, a IB2/NR strain, was tested. When Gen-Probe results, without confirmation by PCA, were considered against culture the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was 93.3%, 97.9%, 82.4% and 99.3% respectively. After resolution of discrepant results by PCA and excluding the patient with previous rectal gonorrhoea Gen-Probe gave a sensitivity, specificity, PPV and NPV of 94.1%, 100%, 100% and 99.3%. The sensitivity of culture was 88.2% (15/17) and

Correlation between culture and Gen-Probe

	Culture positive		Culture negative		Total	
	Rectal	Throat	Rectal	Throat	Rectal	Throat
Gen-Probe positive	14	9	3‡	10§	17	19
Gen-Probe negative	1*	4†	143	138	144	142
Total	15	13	146	148	161	161

*Gen-Probe negative on repeat

†One specimen Gen-Probe positive on repeat

‡All Gen-Probe positive on repeat and confirmed by probe confirmation assay (PCA)

§Nine specimens Gen-Probe positive on repeat and confirmed by PCA.

the NPV was 98.6% (143/145) when measured against the confirmed Gen-Probe results. The difference in sensitivity between Gen-Probe and culture is not significant (χ^2 1.12; $p > 0.2$).

Pharyngeal culture versus Gen-Probe The overall agreement was 91.3% (147/161) with ten of the fourteen discrepancies due to negative cultures. Of the ten patients with positive Gen-Probe results but negative cultures four had rectal gonorrhoea, two were contacts of gonorrhoea, and four had no known risk factors: one of the latter patients was negative on repeat testing. All nine repeat reactive Gen-Probe specimens (seven with RLU values > 1500) gave 97%–99% inhibition in the PCA assay confirming that the specimens contained gonococcal nucleic acid. One of the four Gen-Probe negative (RLU 305 against a cut off of 410) culture positive specimens was positive on repeat testing (RLU 403 against a cut-off of 355) while the others remained negative with initial and repeat RLU values below 300. The corresponding isolates from these four patients gave a positive probe result. When Gen-Probe results, without confirmation by PCA, were considered against culture the sensitivity, specificity, PPV and NPV was 69.2%, 93.9%, 50% and 97.4% respectively. After resolution of discrepant results by PCA Gen-Probe gave a sensitivity, specificity, PPV and NPV of 86.4%, 100%, 100%, and 97.9%. The sensitivity of culture was 59% (13/22) and the NPV 93.9% (139/148) when measured against the confirmed Gen-Probe results. The difference in sensitivity between culture and Gen-Probe is significant ($\chi^2 = 4.13$; $p < 0.05$). The average RLU value for the cut-off was 386 (range 351–450) while the average for the 144 rectal and 142 throat specimens with a negative probe result was 91 and 103 respectively. The average RLU value for a positive result was 20306 (range 403–110 104): this was, however, significantly higher (two sample *t* test; $p = 0.019$) in rectal specimens 31 325 (range 1,705–110 104) than in throat specimens 10 447 (range 403–15 633). The corresponding values for culture negative probe positive rectal and throat specimens were 17 356 (values 1351, 4751, and 46 003) and 16,767 (range 414–65 009) respectively. There was no difference between the mean RLU value for the 51 probe negative throat cultures yielding meningococci on culture (101) and the 83 cultures from which meningococci were not isolated (97): the mean RLU values for the two throat cultures yielding *N perflava* and *M catarrhalis* were 214 and 87 respectively while the single specimen yielding *N lactamica* gave a value of 164.

Discussion

As the Gen-Probe assay is currently recommended only for urogenital specimens performance data for extra-genital specimens are extremely limited.^{10 14} Our results show that Gen-Probe is comparable in sensitivity to rectal culture but is significantly more sensitive than pharyngeal culture and support the earlier sug-

gestion that Gen-Probe provides an alternative to culture for rectal and pharyngeal infection.¹⁴ The sensitivity of culture is not absolute but ranges from 80–95%¹⁸ and may be even lower if certain pre-poured selective media are used.¹⁹ Therefore in order to avoid under-estimating the sensitivity and specificity we re-tested all of the reactive Gen-Probe specimens with a probe competition assay (PCA) although a recent study¹⁷ found that this was not normally necessary for specimens with RLU values > 1500. We also attempted to use the ligase chain reaction²⁰ to corroborate the Gen-Probe results but the specimens were unsuitable for amplification by this method. If the probe results are compared directly with rectal culture without repeat testing or confirmation of the probe result by PCA the sensitivity, specificity, PPV and NPV was 93.3%, 97.9%, 82.4% and 99.3% respectively. However, after confirmation by PCA Gen-Probe gave a sensitivity, specificity, PPV and NPV of 94.1%, 100%, 100% and 99.3%: the sensitivity of culture was reduced to 88.2% and the NPV was 98.6%. Comparing probe results directly with pharyngeal culture gave a sensitivity, specificity, PPV and NPV of 69.2%, 93.9%, 50% and 97.4% respectively which increased to 86.4%, 100%, 100%, and 97.9% after confirmation by PCA: the sensitivity of culture was reduced to 59% and the NPV to 93.9%; the difference in sensitivity between culture and Gen-Probe is significant ($p < 0.05$). The finding that probe positive culture negative pharyngeal results occurred significantly more often ($p < 0.001$) in patients with culture proven rectal gonorrhoea (26.7%) than in those without rectal infection (3.4%) lends epidemiological support to the accuracy of the probe results. False positive probe results due to organisms such as meningococci sharing rRNA sequences to those on which the probe is based are extremely unlikely as the same probe is used in a culture confirmation assay which had absolute specificity when evaluated in an "in-use" assay which included a range of non gonococcal neisseriae.²¹ The finding that Gen-Probe sensitivity was comparable to rectal culture ($p > 0.2$) but significantly higher than throat culture ($p < 0.05$) is in keeping with the significant difference ($p = 0.019$) in the mean RLU value for rectal (31 325) versus throat specimens (10 447) which indicates a higher antigenic load in the rectal specimens. There were three culture positive Gen-Probe negative pharyngeal specimens compared with one culture positive probe negative rectal specimen which is consistent with sampling differences becoming more important when the number of organisms is low.

It is also possible that Gen-Probe detected non-viable antigen in the pharynx. Serological studies²² have suggested that some patients are exposed to pharyngeal gonorrhoea without developing culture-proven pharyngeal infection. *N meningitidis* has been reported to have a possible protective effect against genital gonococcal infection²³ so it is possible that a similar protective effect may occur in the pharynx. Nevertheless a positive result would

appear to indicate exposure to infection and, in the absence of a previously treated infection, could be important in ensuring appropriate partner notification action. There is little information on probe positivity following treatment¹⁰ and further studies are required to monitor the disappearance of gonococcal nucleic acid after therapy.

Our results demonstrating a prevalence of rectal infection of 10.6% for Gen-Probe versus 8.8% for culture and a prevalence of pharyngeal infection of 11.8% for Gen-Probe versus 8.1% for culture provide a baseline by which other centres could use Gen-Probe to evaluate the efficiency of their rectal and pharyngeal cultures. Whenever possible cultures should be obtained from patients with positive probe test results in order to monitor the antibiotic susceptibility and molecular epidemiology of the gonococcal population.

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