Comparison of a DNA probe assay with culture for the detection of *Chlamydia trachomatis*

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Summary. A new DNA probe assay (PACE 2, Gen-probe) was compared with cell culture for the detection of *Chlamydia trachomatis* in 909 women attending the Royal Women's Hospital, Melbourne, Victoria. The DNA probe assay had a sensitivity of 86.2%, a specificity of 99.9% and a positive predictive value of 96.2% in a population with 3.2% prevalence, indicating that it may be a suitable alternative to culture for the detection of *C. trachomatis* in specimens from the genital tract.

Introduction

Chlamydia trachomatis is the most commonly encountered sexually transmitted disease in developed countries; the benchmark method for its diagnosis in the laboratory is cell culture.¹ Other techniques for laboratory diagnosis, such as enzyme immunoassay (EIA) and direct antigen fluorescence (DF), are available but, although more rapid to perform, they are less sensitive than cell culture. Consequently, in populations where the prevalence of chlamydial carriage is low, the positive predictive value of these more rapid techniques becomes less acceptable.²

Recently, a new DNA probe assay (PACE 2; Gen-Probe, San Diego, CA, USA) that has a non-isotopic label and detects ribosomal RNA of *C. trachomatis* has become available commercially. PACE 2 supercedes a previous assay, PACE 1, and has the advantages of being simpler to perform and of greater sensitivity. We compared the DNA probe assay with cell culture in a female population with low prevalence of chlamydial infection.

Materials and methods

The study population was 909 women attending the Royal Women's Hospital, Victoria, Australia between August and December, 1989 inclusive, and who were seen at the Emergency Department as symptomatic patients (539 women) or were asymptomatic patients attending the Pregnancy Advisory Service (PAS) clinic (370 women). At the latter clinic patients are screened routinely for chlamydia before requesting legal termination of pregnancy.

Samples for chlamydial diagnosis

Endocervical swabs were collected from each patient after cervical mucus or pus was wiped away; the order of swabs was randomised. For culture, a cotton-tipped aluminium ENT swab (Medical Wire and Equipment Co., Wilts) was used; it was placed in transport medium, transported on ice to the laboratory and either cultured the same day or stored at -70°C until processed. For DNA probe (gene probe; GP) assay, specimens were collected according to the manufacturer's instructions. A swab was inserted into the endocervical canal and rotated for 10–30 s. The swab was then carefully withdrawn and placed in GP transport medium and transported to the laboratory on the same day. Each specimen was vortex mixed for 10 s, the swab was discarded and the remaining specimen was either stored at 4°C , generally for up to 4 days (7 days was the acceptable maximum) or stored at -70°C until processed.

Cell culture was performed in 48-well cluster trays as previously described³ with the addition of cycloheximide 0.5 mg/L to the cell maintenance medium. The GP assay was performed in batches of 60 tests (including three positive controls and one negative control) according to the manufacturer's instructions. Samples were allowed to come to room temperature and then vortex mixed for 5 s to ensure homogeneity, after which 100 μl was pipetted into an appropriately labelled tube. Probe reagent was prepared by warming probe diluent in a water bath at 60°C for 3–4 min and adding 6 ml to lyophilised probe reagent. This was allowed to stand at room temperature for 2 min and then vortex mixed for 10 s before adding 100 μl to each specimen. After covering the tubes with sealing cards the specimen mixture was incubated in a waterbath at 60°C for 1 h. Concurrently, a separation solution was prepared consisting of 70 ml of activator and 3.2 ml of separation reagent (stable for 6 h). This

separation solution (1 ml) was added to each specimen and allowed to react for 10 min at 60°C, after which tubes were placed on a magnetic separation rack for 5 min at room temperature. The supernates were decanted, and 5 ml of a detergent wash was added and held at room temperature for 20 min. This solution was decanted and the test result for each tube read on a luminometer (Leader 1; Gen-Probe) which measured the number of relative light units (RLU) emitted by the specimen. The test result was calculated and expressed as the difference between the response of the specimen and the mean response of the three negative reference replicates. When this difference was ≥ 300 RLU, the specimen was regarded as positive, i.e., if the mean response of the negative reference was 73, the positive-negative cut-off value was 373 RLU.

Results

The results obtained with 909 specimens tested by GP and cell culture are shown in table I; the sensitivity of the GP assay was 86.2%, with a specificity of 99.9%, a positive predictive value of 96.2% and a negative predictive value of 99.5%. Two of the four specimens which were positive by culture and negative by GP assay gave RLU values very close to the calculated positive-negative cut-off value and, on re-testing, both gave low positive values. The order of collection did not affect the outcome of the result for either test. Specimens that yielded <10 inclusions by culture were all positive by GP assay but generally with low RLU values (table II). High inclusion counts by culture tended to correlate with high RLU values by GP assay.

Table I. Comparison of culture and GP for detection of *C. trachomatis* in endocervical swabs

Culture result	DNA probe result	
	+	-
+	25	4
-	1	879

Table II. RLU values for specimens with low inclusion counts

Specimen no.	Inclusion-forming units/well	RLU value
1	10	1439
2	10	13606
3	6	6806
4	4	596
5	1	654

Discussion

In comparison with culture, the GP assay, PACE 2, was sensitive (86.2%) and specific (99.9%) and performed better than the previously published results for PACE 1, in which sensitivity and specificity were 60% and 80%⁴ and 95% and 98%,⁵ respectively, and for the ¹²⁵I-labelled DNA probe (Gen-Probe), 82.8% and 99.4%.⁶ The primary modification of the PACE 2 format is an additional chemical separation which corresponds with the mechanical separation step in PACE 1. The probes used in both PACE 1 and PACE 2 are synthetic oligonucleotides directed against specific ribosomal RNA targets. As RNA is more abundant than genomic DNA, these tests should be more sensitive than tests with other DNA probes directed against genomic or chlamydial plasmid DNA. Results of other studies with non-commercial DNA-probe technology for the detection of *C. trachomatis* have not differed greatly in sensitivity and specificity from the earlier commercially available GP assays.^{7,8} Our results indicate that the manufacturer's modifications in the PACE 2 format have increased the sensitivity of the test. We were able to detect specimens that contained <10 IFU, whereas with PACE 1, Peterson *et al*⁴ experienced difficulty in detecting <1000 IFU/specimen. The single specimen that gave a positive GP signal (3471 RLU) but was culture negative was also negative by DF. This may have been a false positive GP result, or may have resulted from sampling variation for each test from that individual patient.

As with EIA, PACE 2 is suited to the batch testing of large numbers of specimens. We found the assay simple and quick to perform (60 tests could be performed in 3 h with a total "hands on" time of 90 min) and in the vast majority of cases gave very clear results, well above or below the cut-off level. Two specimens yielded levels close to the cut-off and on re-testing gave positive results. Consequently, we believe that a borderline zone needs defining with a recommendation that, for specimens which fall into this area after repeat testing, a fresh specimen should be obtained and tested by another method.

The GP assay does not require stringent transport and storage conditions. The manufacturers recommend limits of transport and storage temperature between 2°C and 25°C for up to 7 days, outside which specimens may be stored frozen at -20°C or -70°C. These are similar to the conditions required for EIA techniques and are far less demanding than those for tissue culture, for which specimens may be stored and transported at 4°C only for up to 24 h, beyond which freezing at -70°C is mandatory. However, GP is more costly in materials than EIA, although it is still considerably cheaper to perform than tissue culture with conventional coverslips and vials when the labour cost is taken into account. In our laboratory, tissue culture is streamlined by the use of multiwell trays which greatly reduce labour costs.³ Under these

circumstances the total cost of GP and tissue culture would be similar.

In reports of comparisons of each of GP, EIA and DF with culture as the reference method, all of the rapid techniques have yielded similar results.⁹⁻¹⁴ Most studies have been performed in patient populations with a higher prevalence of *C. trachomatis* than ours. As the positive predictive value of a given test varies directly with the prevalence of disease in the population studied, it has been suggested that non-culture methods for the detection of *C. trachomatis* are less

suitable than culture in populations of low chlamydial prevalence.^{2,12} As a positive predictive value of 96.2% was found for PACE 2 when compared with culture in a population with 3.2% prevalence of *C. trachomatis*, it must be considered a suitable alternative to culture for the detection of *C. trachomatis* in genital specimens, especially when transport of specimens is difficult.

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References

1. Ridgway GL. The laboratory diagnosis of chlamydial infection. In: Oriel D, Ridgway G, Schachter J *et al.* (eds) *Chlamydial infections*. Cambridge, Cambridge University Press. 1986: 539-549.
2. Lipkin ES, Moncada JV, Shafer M-A, Wilson TE, Schachter J. Comparison of monoclonal antibody staining and culture in diagnosing cervical chlamydial infection. *J Clin Microbiol* 1986; **23**: 114-117.
3. Lees MI, Newman DM, Garland SM. Simplified culture procedure for large-scale screening for *Chlamydia trachomatis* infections. *J Clin Microbiol* 1988; **26**: 1428-1430.
4. Peterson EM, Oda R, Alexander R, Greenwood JR, de la Maza LM. Molecular techniques for the detection of *Chlamydia trachomatis*. *J Clin Microbiol* 1989; **27**: 2359-2363.
5. Woods GL, Young A, Scott JC, Blair TMH, Johnson AM. Evaluation of a nonisotopic probe for detection of *Chlamydia trachomatis* in endocervical specimens. *J Clin Microbiol* 1990; **28**: 370-372.
6. LeBar W, Herschman B, Jemal C, Pierzchala J. Comparison of DNA probe, monoclonal antibody enzyme immunoassay, and cell culture for the detection of *Chlamydia trachomatis*. *J Clin Microbiol* 1989; **27**: 826-828.
7. Pao CC, Lin S-S, Yang T-E, Soong Y-K, Lee P-S, Lin J-Y. Deoxyribonucleic acid hybridization analysis for the detection of urogenital *Chlamydia trachomatis* infections in women. *Am J Obstet Gynecol* 1987; **156**: 195-199.
8. Horn JE, Quinn T, Hammer M, Palmer L, Falkow S. Use of nucleic acid probes for the detection of sexually transmitted infectious agents. *Diagn Microbiol Infect Dis* 1986; **4** Suppl 3: 101S-109S.
9. Mumtaz G, Mellars BJ, Ridgway GL, Oriel JD. Enzyme immunoassay for the detection of *Chlamydia trachomatis* antigen in urethral and endocervical swabs. *J Clin Pathol* 1985; **38**: 740-742.
10. Mahoney J, Castriciano S, Sellors J *et al.* Diagnosis of *Chlamydia trachomatis* genital infections by cell culture and two enzyme immunoassays detecting different chlamydial antigens. *J Clin Microbiol* 1989; **27**: 1934-1938.
11. Tilton RC, Judson FN, Barnes RC, Gruninger RP, Ryan RW, Steingrimsson O. Multicenter comparative evaluation of two rapid microscopic methods and culture for detection of *Chlamydia trachomatis* in patient specimens. *J Clin Microbiol* 1988; **26**: 167-170.
12. Lees M, Newnan D, Garland SM. Detection of *Chlamydia trachomatis* by direct immunofluorescence and culture in genital specimens of symptomatic and asymptomatic women. *Venereology* 1990; **3**: 28-31.
13. Smith JW, Rogers RE, Katz BP *et al.* Diagnosis of chlamydial infection in women attending antenatal and gynecologic clinics. *J Clin Microbiol* 1987; **25**: 868-872.
14. Chernesky MA, Mahoney JB, Castriciano S *et al.* Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic men and women. *J Infect Dis* 1986; **154**: 141-148.