SHORT COMMUNICATION

Detection of *Chlamydia trachomatis* in Clinical Specimens by Nucleic Acid Spot Hybridization

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(Received 22 November 1984)

A nucleic acid spot hybridization assay was used to detect *Chlamydia trachomatis* DNA. The hybridization probes included DNA isolated from elementary bodies of lymphogranuloma venereum (LGV) strains and cloned fragments of both chromosomal and plasmid DNA. The sensitivity of the test was in the range 10 to 100 pg homologous DNA and 10 *in vitro* infected cells. Cross-reactivity with bacterial DNA was avoided when purified chlamydia-specific DNA fragments were used as probes. *C. trachomatis* was detectable in most of the clinical specimens with large amounts of infectious particles. Also some isolation-negative specimens gave a positive signal in the test.

INTRODUCTION

Chlamydia trachomatis is currently the most common cause of sexually transmitted diseases in a majority of western countries. Definitive diagnosis of chlamydial infections is usually made by isolation of infectious organisms in cell culture (Darougar *et al.*, 1971) or by direct demonstration of specific antigens in inflammatory exudates using monoclonal antibody staining (Tam *et al.*, 1984). Both methods, although specific and sensitive, need microscopy for evaluation of test results and are therefore time consuming and require a trained specialist.

We recently described a spot hybridization test for detection of chlamydial DNA (Hyypiä *et al.*, 1984). In the present report we have further evaluated the sensitivity and specificity of the test, and used it to detect *C. trachomatis* DNA in clinical specimens.

METHODS

Specimens. McCoy cells, treated with 1 µg cycloheximide ml⁻¹, were infected with *Chlamydia trachomatis* LGV strains L1 (440-L), L2 (434-B) and L3 (404-L) at a m.o.i. of about 1·0 IFU (inclusion-forming unit) per cell. The cells were grown for 3 d, at which time 80–90% of them showed visible inclusions, harvested and stored at –70 °C until tested. Prior to the test, the cells were treated with 0·1 mg proteinase K ml⁻¹ (Merck) for 1 h at 37 °C, denatured by boiling in 0·3 M-NaOH, chilled on ice and neutralized with HCl.

Control microbial strains were obtained from routine isolations (Department of Medical Microbiology, University of Turku) and identified by standard procedures (Lennette *et al.*, 1980). The strains included isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus viridans*, *Corynebacterium* sp., *Lactobacillus* sp., *Bacteroides fragilis* and *Candida albicans*. The micro-organisms were grown on plates or in liquid medium. For the test, a suspension of each of the microbes was boiled at an alkaline pH, chilled on ice and neutralized, as described above.

Male urethral and female cervical specimens were taken with cotton-tipped swabs into transport medium [0·2 M-sucrose, 0·02 M-sodium phosphate buffer containing 3% (v/v) foetal calf serum, 2·5 IU nystatin ml⁻¹ and 50 µg gentamicin ml⁻¹] and sent to our laboratory for isolation of *C. trachomatis*. Specimens were stored at –20 °C

until tested. The isolation was carried out in irradiated McCoy cells as described by Terho (1978). For the hybridization assay, the specimens were treated with proteinase K and either extracted with phenol, ethanol precipitated and denatured as above or denatured directly on the filter (Totten *et al.*, 1983).

Hybridization probes. Purification of *C. trachomatis* DNA from elementary bodies and characterization of the cloned L2 plasmid have been described previously (Hyypä *et al.*, 1984). For the hybridization experiments, the hybrid plasmid was digested with *Bam*HI (Boehringer-Mannheim) and the L2 plasmid insert was recovered from agarose gels by use of a DEAE-cellulose membrane (Dretzen *et al.*, 1981).

Cloned chromosomal probes were constructed as follows. DNA purified from elementary bodies of strain L1, and pBR322 plasmid DNA were digested with *Bam*HI and ligated using T4 DNA ligase (Boehringer-Mannheim). The recombinant DNA was used to transform *E. coli* HB101. The ampicillin resistant, tetracycline sensitive colonies were screened by colony hybridization using an L1 DNA probe. Recombinant plasmid DNA was purified from one of the positive colonies and used as a probe in the spot hybridization test.

For the hybridization assay, the probes were labelled with ^{32}P in a nick translation reaction (Rigby *et al.*, 1977) to a specific activity of $10\text{--}100 \times 10^6$ c.p.m. μg^{-1} .

Spot hybridization test. The specimens were either spotted onto nitrocellulose filters (Schleicher & Schüll, Dassel, FRG) after denaturation by boiling in alkali, or were denatured *in situ* after spotting onto the filters. The filters were baked for 1 h at 80°C and hybridization was carried out as described by Wahl *et al.* (1979). The radioactivity bound onto the filters was revealed by autoradiography.

RESULTS AND DISCUSSION

Sensitivity and specificity of the hybridization test

The sensitivity and specificity of the spot hybridization assay were evaluated using purified homologous and control DNA, and *in vitro* infected and control McCoy cells (Fig. 1). When whole DNA from L1 elementary bodies was used as a probe the sensitivity was 10–100 pg L1 DNA and 10 infected cells. No binding of the probe to herpes simplex virus DNA or to pBR322 plasmid DNA was observed. Large amounts of uninfected cells caused a slight background signal. Similar sensitivity and specificity were observed with cloned chromosomal and plasmid DNA probes with the exception that pBR322 DNA on the filter was detected at a sensitivity of 10–100 pg.

The sensitivity obtained here (less than 100 pg, corresponding to about 10^5 molecules) is somewhat better than that obtained by a recently described sandwich hybridization assay for *C. trachomatis* (Palva *et al.*, 1984). This may be explained by the differing test principle or the different radiolabelling methods. The slight background reaction of the probes with large amounts of uninfected cells indicates that these should be included routinely as a control in the test.

The cross-reactivity of the probes with various micro-organisms was tested because urethral and cervical specimens are sometimes contaminated with bacteria and *Candida*. Whole L1 chromosomal DNA showed reactivity with large amounts of some enterobacterial and gonococcal DNA when the autoradiographs were overexposed (Fig. 2, A). When the purified L2 plasmid DNA insert was used as a probe no reaction occurred even after longer exposures (Fig. 2, B). Because the large amounts of microbes sufficient to cause detectable background reactions are not usually present in swab specimens, the whole chromosomal DNA probe was used in the further assays.

Detection of C. trachomatis in clinical specimens

In order to see whether the clinical specimens would contain enough chlamydial DNA to give a positive signal in the spot hybridization assay, we tested eight specimens with large amounts of infectious particles and eight isolation-negative specimens. The whole L1 chromosomal DNA probe recognized seven of the positive specimens, and one of the negative samples was also scored positive (Fig. 3).

In the second part of the study with clinical specimens, 30 isolation-positive and 30 isolation-negative specimens were tested directly after proteinase treatment and *in situ* denaturation. Ten of the isolation-positive samples gave a signal which exceeded the signal of any of the negatives (Fig. 4). Overall, 24 of the 30 positives and 7 of the negatives gave a signal in the test.

Fig. 1. Detection of chlamydial DNA by spot hybridization using whole L1 DNA probe. Various amounts of purified DNA or cells, treated with proteinase K, were denatured, applied to nitrocellulose filters and detected by a ^{32}P -labelled probe. The results were revealed by autoradiography. 1, Whole L1 DNA; 2, herpes simplex virus DNA; 3, pBR322 DNA; 4, McCoy cells; 5, L1 infected McCoy cells; 6, L2 infected McCoy cells; 7, L3 infected McCoy cells.

Fig. 2. Specificity of chlamydial probes. DNA or the cells were boiled in alkali, neutralized, applied to nitrocellulose filters and detected by ^{32}P -labelled probes. A, Whole L1 chromosomal DNA probe; B, L2 plasmid insert. 1, L1 infected McCoy cells; 2, McCoy cells; 3–5, *Escherichia coli*; 6, *Klebsiella pneumoniae*; 7, *Proteus mirabilis*; 8, *Citrobacter freundii*; 9, *Enterobacter aerogenes*; 10, *Salmonella typhimurium*; 11, *Yersinia enterocolitica*; 12, *Pseudomonas aeruginosa*; 13, *Gardnerella vaginalis*; 14, *Neisseria gonorrhoeae*; 15, *Staphylococcus aureus*; 16, *Streptococcus agalactiae*; 17, *Strep. faecalis*; 18, *Strep. viridans*; 19, *Corynebacterium* sp.; 20, *Lactobacillus* sp.; 21, *Bacteroides fragilis*; 22, *Candida albicans*.

Our findings confirm the earlier observations of Palva *et al.* (1984) that nucleic acid hybridization assays can be used to demonstrate the presence of *C. trachomatis* in clinical specimens. The sensitivity of the probe can be selected so that cross-reactivity with other microbial DNA usually present in the specimens does not occur. The problem, however, is the sensitivity. Some of the positive samples routinely taken for isolation do not contain sufficient amounts of specific DNA sequences to give a signal for specific diagnosis. Therefore either more sensitive probe systems or better sampling methods are required.

Fig. 3. Detection of chlamydial DNA in clinical specimens by spot hybridization. Eight isolation-positive (1-8) and eight isolation-negative (9-16) specimens were treated with proteinase K and phenol extracted. DNA was ethanol precipitated, denatured, applied to a nitrocellulose filter and detected by a ^{32}P -labelled whole L1 chromosomal DNA probe.

Fig. 4. Detection of chlamydial DNA in clinical specimens. 30 isolation-positive (1-30) and 30 isolation-negative (31-60) specimens were treated with proteinase K and spotted onto a nitrocellulose filter. DNA was denatured on the filter and detected by a ^{32}P -labelled whole L1 chromosomal DNA probe.

The excellent technical assistance of Marita Maaronen is acknowledged. This study was supported by grants from the Emil Aaltonen Foundation, the Yrjö Jahnsson Foundation, the Sigrid Juselius Foundation and a Program Development Award from Indiana University School of Medicine to S.H.L.

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