

Monitoring of *Chlamydia trachomatis* infections after antibiotic treatment using RNA detection by nucleic acid sequence based amplification

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Abstract

Aim—To investigate the value of RNA detection by nucleic acid sequence based amplification (NASBA) for the monitoring of *Chlamydia trachomatis* infections after antibiotic treatment.

Methods—Cervical smears (n = 97) and urine specimens (n = 61) from 25 *C trachomatis* positive female patients were analysed for the presence of *C trachomatis* 16S ribosomal RNA (rRNA) by NASBA and *C trachomatis* plasmid DNA by the polymerase chain reaction (PCR) before and up to five weeks after antibiotic treatment.

Results—*Chlamydia trachomatis* RNA was found in all cervical smears taken before antibiotic treatment (n = 24) and in two smears taken one week after antibiotic treatment; no *C trachomatis* RNA was detected after two weeks or more. In contrast, *C trachomatis* DNA was found in all such specimens before treatment, and 21 of 25, six of 21, and five of 20 smears were found to be positive at one, two, and three weeks after treatment, respectively. After four weeks, only one of six smears was positive, and this smear had been negative in the two preceding weeks. Of the 61 urine samples investigated, *C trachomatis* DNA and *C trachomatis* RNA were found in all before treatment (n = 15), whereas one week after treatment four of 15 were *C trachomatis* DNA positive and *C trachomatis* RNA was detected in one sample only.

Conclusions—These data show that RNA detection by NASBA can be used successfully to monitor *C trachomatis* infections after antibiotic treatment. Furthermore, it might be possible to use urine specimens as a test of cure because neither *C trachomatis* DNA or RNA could be detected two weeks or more after treatment.

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Keywords: *Chlamydia trachomatis*; nucleic acid sequence based amplification; antibiotic treatment

Chlamydia trachomatis, an obligate intracellular microorganism, is the most prevalent sexually transmitted agent in the USA and western Europe.^{1,2} The urogenital diseases, caused mainly by *C trachomatis* serovars D-K, include urethritis, endometritis, cervicitis, and pelvic inflammatory disease. In addition, asymptomatic infections could, when untreated, result

in ectopic pregnancy and tubal infertility.³ In general, treatment of these infections with antibiotics (such as doxycycline and azitromycin) is effective.^{4,5} However, in some cases, recurrent *C trachomatis* infections have been seen after antibiotic treatment.^{4,6} This could be a result of reinfection or it may represent persistent disease. Although these persistent cases have been described in vitro,^{7,8} no direct evidence of *C trachomatis* persistence in humans has been reported to date.

To monitor the efficiency of antibiotic treatment of *C trachomatis*, both cell culture and DNA amplification techniques (the polymerase chain reaction (PCR) and the ligase chain reaction (LCR)) have been used. After antibiotic treatment, *C trachomatis* DNA is still found for up to three weeks,^{9,10} although cell culture results are negative one week after antibiotic treatment.^{5,6,9-11} This might result from the detection of DNA from non-viable *C trachomatis* organisms by PCR after antibiotic treatment. Therefore, detection of viable microorganisms after such treatment by cell culture is clinically more relevant than DNA detection by the PCR. However, cell culture can be rather insensitive compared with PCR because of the high demands on sampling of the clinical material and subsequent transport to the laboratory to retain viability of the microorganisms. Alternatively, detection of RNA sequences also measures biological activity and might be an indicator of gene expression and cell viability. Recently, the sensitive isothermal RNA amplification method, that is, NASBA has made its appearance and has been used successfully for the detection of *C trachomatis* in cervical smears and urine specimens.¹² With this technique and using L2 serovar dilution lines, it was possible to detect 0.001 inclusion forming units (IFU) v 0.01 IFU with PCR.¹² Recently, *C trachomatis* detection in urine, a non-invasive sampling method, has been introduced successfully for the routine diagnosis of *C trachomatis* infections. The detection of *C trachomatis* DNA in urine by PCR^{13,14} and LCR^{15,16} is highly sensitive and specific compared with cell culture and enzyme immunoassay (EIA). This suggests that urine could possibly be used for the detection of either *C trachomatis* DNA by PCR or *C trachomatis* RNA by NASBA for the monitoring of *C trachomatis*, instead of using cell culture of cervical smears.

In our study, we investigated the value of RNA detection by NASBA for the monitoring

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of *C trachomatis* infections after antibiotic treatment. Therefore, both cervical smears and urine specimens from *C trachomatis* positive women were analysed for the presence of both *C trachomatis* DNA by PCR and *C trachomatis* RNA by NASBA before and up to five weeks after antibiotic treatment. Furthermore, NASBA using such specimens was evaluated for its use as a test of cure.

Materials and methods

SPECIMEN COLLECTION

Cervical smears (n = 97) were obtained from 25 symptomatic patients attending the department of Gynaecology, OLVG Hospital in Amsterdam for a variety of gynaecological complaints. In addition, urine samples (n = 61) were obtained from 16 patients. These patients, who were found to be positive for *C trachomatis* by EIA (Chlamydiazyme; Abbott Diagnostic Division, Amstelveen, The Netherlands), were treated with 100 mg doxycycline twice a day for one week and were monitored for five weeks. Cervical smears were collected before antibiotic treatment (t = 0; n = 24, one cervical smear was not obtained) and one week (t = 1; n = 25), two weeks (t = 2; n = 21), three weeks (t = 3; n = 20), four weeks (t = 4; n = 6), and five weeks (t = 5; n = 1) after the start of treatment. In addition, 15, 15, 13, 13, four, and one urine specimens, respectively, were collected at these time points. Cervical smears were collected with cervical brushes (Rover BV, Oss, The Netherlands) and placed in 3 ml of lysis buffer (50 mM Tris/HCl pH 6.4, 20 mM EDTA, 1.3% wt/vol Triton X-100, 5.25 M guanidinium thiocyanate (GuSCN)). After vortexing, the samples were transported to the laboratory at room temperature on the day of collection. The cervical brushes were removed after vortexing and the samples were diluted further with 7 ml lysis buffer and stored at -80°C.

First void urine samples were collected into sterile containers (content 25 ml), transported at room temperature, and processed immediately by centrifuging 1.5 ml at 16 000 ×g (Merck, Eppendorf Centrifuge 5415C; Merck, Amsterdam, The Netherlands) for 10 minutes. Supernatants were discarded and pellets stored at -80°C.

PRETREATMENT OF CLINICAL SPECIMENS FOR NASBA/PCR

Nucleic acid (DNA/RNA) released in the lysed cervical cell suspension was bound to activated silica (70 µl of silica, 1 g/ml suspension in 0.1 N HCl; Fluka Chemie AG, Buchs, Switzerland) during 10 minutes of incubation at room temperature.¹⁷ After washing and drying of the silica, the nucleic acid was eluted in 75 µl of distilled water. For DNA/RNA purification from urine, 1 ml of lysis buffer was added to the 1.5 ml urine pellets. Subsequently, the silica based nucleic acid isolation method was performed as described for the cervical smears.

DETECTION BY PCR

Each specimen was subjected to human β globin PCR to check the suitability of the

processed sample for PCR purposes, as described previously.³ Plasmid specific primers (PL6.1: 5'-AGAGTACATCGGTCAACGA-3' and PL6.2: 5'-TCACAGCGGTTGCTCGAAGCA-3'; synthesised by Perkin Elmer, Nieuwerkerk a/d Amstel, The Netherlands)¹² were used for PCR amplification. As a positive control, a 10 fold dilution series of *C trachomatis* L2 nucleic acid was used as described previously.¹²

These PCRs were performed as described previously^{9 18} in a total volume of 50 µl, containing 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 25 pmol of each primer, 1 U of Taq polymerase (Amplitaq; Cetus, Emeryville, California, USA), and 5 µl of isolated nucleic acid. Amplification by the PCR consisted of denaturation for four minutes at 94°C, followed by 40 cycles of amplification: each cycle consisted of one minute at 95°C, one minute at 55°C, and 1.5 minutes at 72°C. The final elongation step was extended for another four minutes. The amplified DNA was analysed by 1.5% agarose gel electrophoresis and ethidium bromide staining. Additional Southern blot analysis was performed by transfer of the DNA on to positively charged nylon membranes (Qiabran nylon plus; Qiagen GmbH, Hilden, Germany) with diffusion blotting in 0.5 N NaOH, 0.6 M NaCl. Membranes were preincubated for two hours at 55°C in hybridisation solution (0.5 M sodium phosphate, pH 7.4, 7% sodium dodecyl sulphate (SDS), 1 mM EDTA). Afterwards, a specific ³²P-dATP end labelled oligonucleotide probe (OT1570: 5'-CGTGCGGGGTTATCTTAAAGGGAT-3') was added and hybridisation was carried out at the same temperature overnight. Subsequently, the membranes were washed three times using 3× SSC/0.5% SDS (1× SSC: 0.15 M NaCl, 0.015 M sodium citrate) for 30 minutes at 55°C. Autoradiography was performed at -80°C with Kodak Royal X-Omat films and intensifying screens.

DETECTION BY NASBA

Primers specific for *C trachomatis* 16S rRNA (OT1257: 5'-AATTCTAATACGACTCAC TATAGGGCTCGGATGCCCAAATATCGC CACA-3' = T7-primer and OT1252: 5'-GATGAGGCATGCAAGTCGGAA-3') were synthesised and purified as described previously.¹⁹ To exclude either the presence of inhibitory factors in the amplification or inefficient sample preparation an internal standard, which is not amplified by NASBA in the presence of wild-type *C trachomatis* 16S ribosomal RNA (rRNA), was added to cervical smears and urine specimens before sample preparation as described previously.¹² From a wild-type 16S rRNA stock¹² a 10 fold dilution series was made from 2 × 10³ to 2 × 10⁻¹ molecules/µl, from which 5 µl was used for NASBA, generating positive controls from 10⁴ to 10⁰ target molecules in the final NASBA reaction mixtures. NASBA amplifications were carried out as described by Kievits *et al* with minor modifications.¹⁹ Reactions were performed in a 20 µl reaction mixture containing 40 mM Tris

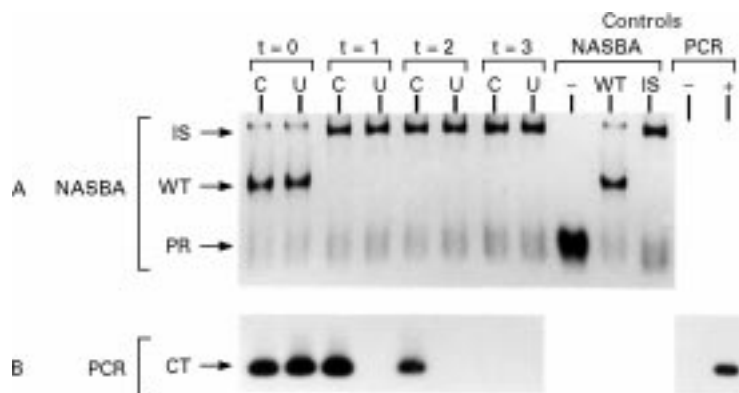


Figure 1 Detection of *Chlamydia trachomatis* in cervical smears and corresponding urine specimens. (A) *Chlamydia trachomatis* and NASBA products detected by ELGA before antibiotic treatment ($t = 0$) and 1, 2, and 3 weeks after treatment. (In the WT *C trachomatis* NASBA positive samples, an amplicon of unknown origin, which is slightly longer than the IS, was observed as described previously).¹² (B) Corresponding *C trachomatis* PCR products detected by Southern blot hybridisation. C, cervical smear; ELGA, enzyme linked gel assay; IS, 16S rRNA NASBA internal standard (285 nucleotides); PR, probe; U, urine; WT, wild-type 16S rRNA (151 nucleotides).

pH 8.5 (Sigma, Poole, Dorset, UK), 12 mM MgCl₂ (Sigma), 70 mM KCl (Mallinckrodt Baker B.V., Deventer, The Netherlands), 5 mM dithiothreitol (DTT; Sigma), 15% vol/vol dimethyl sulphoxide (DMSO; Sigma), 1 mM of each deoxy-nucleotide triphosphate (dNTP; Pharmacia Biotech Benelux, Roosendaal, The Netherlands), 2 mM rATP/rUTP/rCTP and 1.5 mM rGTP (Pharmacia), 0.5 mM ITP (Boehringer, Mannheim, Germany), 2.1 µg bovine serum albumin (BSA; Boehringer), 0.08 U RNaseH (Pharmacia), 6.4 U avian myeloblastosis reverse transcriptase (AMV-RT; Seikagaku America Inc, Ijamsville, Maryland, USA), 32 U T7 RNA polymerase (Pharmacia), 0.2 µM of each primer, and 5 µl of isolated nucleic acid. Reactions were performed at 41°C for 90 minutes and were stopped by placing the reaction mixtures on ice. The amplified product was detected by means of an enzyme linked gel assay (ELGA).²⁰ Precautions similar to those for PCR were taken to prevent contamination.

For detection by ELGA, 3 µl of RNA from NASBA, 1 µl of 6× SSC, 1 µl of layermix, and 1 µl of target specific horseradish peroxidase (HRP) probe (5'-NH₂AGCAATTGTTT CGGCAATTGTTT-3') at 3.3×10^{-8} M were incubated for 15 minutes at 45°C. An aliquot of 3 µl of this solution was used for electrophoresis on a 7% polyacrylamide/bisacrylamide gel containing 0.04% dextran sulphate. The gel was stained on a rotation shaker, using a tetra methyl benzidine (TMB) substrate solution (0.5 ml of TMB/DMSO (5 mg/ml), 24.5 ml of TMB buffer (10 mM citrate, 10 mM EDTA, pH 4.8, and 2.5 µl of H₂O₂)), until the blue bands became clearly visible.

CELL CULTURE

To compare PCR and NASBA results with those of cell culture, five additional culture positive cervical smears were included. After removing cervical mucous, one swab (cotton tipped with aluminium shaft; Hospidex, Nieuwkoop, The Netherlands) was taken for isolation of *C trachomatis* in cell culture. The swab was

placed in 2 ml chlamydia transport medium 4SP (0.4 M sucrose phosphate buffer, pH 7.2, supplemented with 10% fetal calf serum and antibiotics) and stored at -80°C until cultured. Cell culture was performed according to routine procedures. Briefly, HeLa 229 cells (ATCC CCL 2.1) were maintained in Iscove's modified Dulbecco's medium (Gibco Life Sciences, Breda, The Netherlands) supplemented with 10% fetal calf serum and antibiotics. One day old monolayers of HeLa 229 cells were used for the isolation of *C trachomatis*. All monolayers were pretreated with 30 µl/ml DEAE dextran in Hank's balanced salt solution for 15 minutes at room temperature. Clinical specimens were thawed rapidly in a water bath at 37°C and vortexed vigorously. Then, 0.3 ml was inoculated on to a monolayer on a glass cover slip in a shell vial. The vials were centrifuged for one hour at 4800 ×g at 25°C and incubated at 37°C with 5% CO₂ for three days. *Chlamydia trachomatis* growth was assessed by staining with fluorescein labelled monoclonal antibodies (PathoDx assay; Diagnostic Products Corporation, Los Angeles, USA). For PCR and NASBA assays, 0.5 ml aliquots of the 4SP samples were centrifuged at 16 000 ×g for 30 minutes. Subsequently, the pellet was resuspended in lysis buffer (L6) and nucleic acid was isolated with the silica based isolation method already described.

The sensitivity of cell culture was compared with NASBA and PCR using serial dilutions. Five clinical isolates were cultured as described above until 25–50% was infected with *C trachomatis*. Isolates were resuspended in 2 ml Iscove's modified Dulbecco's medium and sonicated. Serial dilutions of 300 µl were made from 10⁻³ to 10⁻¹⁰. Subsequently, 100 µl was used for cell culture and 100 µl was used to isolate the nucleic acid obtained in a 200 µl eluate. The remaining 100 µl was stored at -80°C as a backup. Cell culture results were evaluated after two days using the PathoDx assay (Diagnostic Products Corporation). NASBA and PCR were performed using 5 µl nucleic acid as described above.

Results

DETECTION OF *C TRACHOMATIS* RNA AND DNA IN CERVICAL SMEARS

A comparison of RNA detection using NASBA and DNA detection using PCR was made on cervical smears (n = 97) of *C trachomatis* EIA positive patients (n = 25) followed up for four weeks after antibiotic treatment. All cervical smears tested were positive for β globin PCR, indicating that no inhibition of DNA amplification occurred.

Figure 1 shows the results of *C trachomatis* specific RNA and DNA detection in cervical smears from a representative patient before and up to three weeks after antibiotic treatment. Before antibiotic treatment, both *C trachomatis* RNA as detected by 16S rRNA NASBA and visualised by ELGA (151 nucleotides; fig 1A) and *C trachomatis* DNA as detected by plasmid PCR (130 base pairs; fig 1B) are present. After antibiotic treatment, *C trachomatis* RNA is no longer detected, whereas the NASBA internal

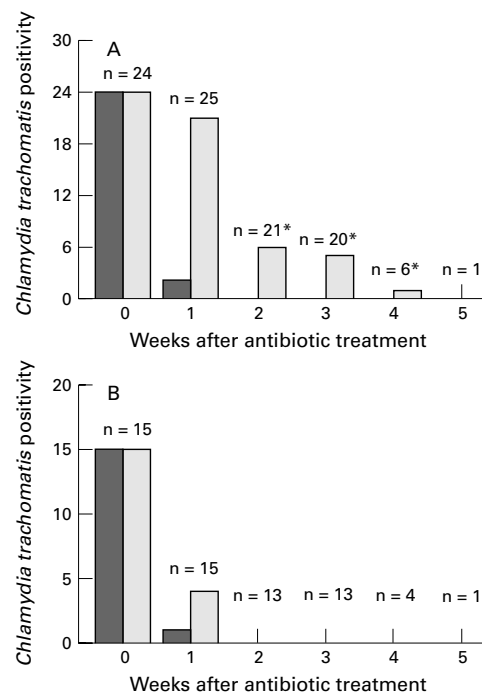


Figure 2 (A) The number of *Chlamydia trachomatis* positive samples in cervical smears and (B) urine specimens before and after antibiotic treatment. *Chlamydia trachomatis* infections were analysed by plasmid DNA PCR (light grey bars) and 16S rRNA NASBA (dark grey bars). This figure also contains the four cases showing fluctuating *C trachomatis* DNA PCR results, which are described in detail in table 1. *, contains sample(s) in which the preceding smear was *C trachomatis* DNA negative.

standard (285 nucleotides) is clearly visible. This was the case in all cervical smears negative for chlamydia, which indicates that there was no inhibition of amplification and that sample preparation was adequate. *Chlamydia trachomatis* DNA was still detectable up to two weeks after antibiotic treatment, as shown by Southern blot hybridisation (fig 1B).

Results of the *C trachomatis* DNA and RNA detection in all of the cervical smears tested are summarised in fig 2A. Before antibiotic treatment (t = 0) *C trachomatis* RNA and DNA could be detected in all cervical smears (n = 24). One week after the start of antibiotic treatment (t = 1), *C trachomatis* RNA was present in only two of 25 cervical smears, although *C trachomatis* DNA could still be detected in 21 of 25 such specimens. From two weeks after treatment (t = 2), *C trachomatis* RNA was no longer detected (fig 2A). In contrast, at week 2 (t = 2), week 3 (t = 3), week 4 (t = 4), and week 5 (t = 5) *C trachomatis* DNA was present in six of 21, five of 20, one of six,

and none of one cervical smears, respectively (fig 2A). In four of these patients, reappearance of *C trachomatis* DNA was seen in their smears even though the preceding smear had been negative (table 1). However, these cases were only weakly positive after Southern hybridisation and no *C trachomatis* RNA could be detected by NASBA.

DETECTION OF *C TRACHOMATIS* RNA AND DNA IN URINE SPECIMENS

Comparison of RNA detection using NASBA and DNA detection using PCR of *C trachomatis* was also performed on first void urines (n = 61) from 16 patients with *C trachomatis* EIA positive cervical smears. All urine specimens tested were positive for β globin PCR, indicating that no inhibition of DNA amplification had occurred.

Results of *C trachomatis* RNA and DNA detection in urine specimens from a representative patient before and up to three weeks after antibiotic treatment are shown in fig 1, along with the results of the corresponding cervical smears. Before treatment, both *C trachomatis* RNA as detected by 16S rRNA NASBA (151 nucleotides; fig 1A) and *C trachomatis* DNA as detected by plasmid PCR (130 bp; fig 1B) were present. After antibiotic treatment, *C trachomatis* RNA or DNA were not detected (fig 1A and B), whereas the NASBA internal standard (285 nucleotides) was clearly visible.

The results of *C trachomatis* DNA and RNA detection in all the urine specimens tested are summarised in fig 2B. Before antibiotic treatment, *C trachomatis* RNA and DNA could be detected in all urine specimens (n = 15). One week after antibiotic treatment, *C trachomatis* RNA was seen in only one of 15 urine specimens, although *C trachomatis* DNA was still detected in four of 15 specimens. This included one urine specimen that initially showed no amplification by NASBA, but a new nucleic acid isolation of the same urine specimen showed amplification of the 16S rRNA internal standard, indicating inefficient isolation the first time. At weeks 2, 3, 4, and 5 neither *C trachomatis* RNA nor DNA could be detected.

COMPARISON OF CELL CULTURE AND *C TRACHOMATIS* RNA AND DNA DETECTION USING CERVICAL SMEARS

The comparison between cell culture, RNA detection using NASBA, and DNA detection using PCR was performed on cervical smears (n = 19) of *C trachomatis* cell culture positive

Table 1 NASBA and PCR results from patients with reappearing *Chlamydia trachomatis* DNA

Patient	t (0)		t (1)		t (2)		t (3)		t (4)		t (5)	
	Cx N,P	Ur N,P	Cx N,P	Ur N,P	Cx N,P	Ur N,P	Cx N,P	Ur N,P	Cx N,P	Ur N,P	Cx N,P	Ur N,P
1	++	++	-+	-	-	-	-	-	-+	-	-	-
2	++	NA	-+	NA	-	NA	-+	NA	NA	NA	NA	NA
3	++	NA	-	NA	-+	NA	-	NA	NA	NA	NA	NA
4	++	NA	-	NA	-	NA	-+	NA	NA	NA	NA	NA

t (0), before treatment; t (1), t (2), t (3), t (4), and t (5): 1, 2, 3, 4, and 5 weeks after antibiotic treatment, respectively.

Cx, cervical smear; Ur, first void urine specimen; P, *Chlamydia trachomatis* plasmid PCR followed by Southern blot hybridisation; N, 16S rRNA NASBA followed by enzyme linked gel assay (ELGA); NA, specimen not available.

patients (n = 5) followed up for four weeks after antibiotic treatment. All cervical smears tested were positive for β globin PCR and the NASBA internal standard was clearly visible in all *C trachomatis* RNA negative cervical smears, indicating that no inhibition of amplification had occurred.

Before antibiotic treatment, cell culture was positive for all patients. Also, both *C trachomatis* RNA as detected by 16S rRNA NASBA and *C trachomatis* DNA as detected by plasmid PCR were present. One week after the start of the antibiotic treatment all cervical smears were negative by cell culture for *C trachomatis*. On the other hand, *C trachomatis* RNA was detected in one cervical smear, and *C trachomatis* DNA was detected in all five such specimens one week after the start of the antibiotic treatment. Two, three, and four weeks after antibiotic treatment (no specimens were obtained at weeks 2 and 3 from three patients) neither *C trachomatis* DNA nor RNA was detected.

To compare the sensitivity of cell culture with NASBA and PCR in a reconstruction experiment, serial dilutions of five clinical isolates were made and subsequently subjected to cell culture, PCR, and NASBA analysis. The following dilutions could be detected by cell culture, PCR, and NASBA, respectively—series 1: 10^{-6} , 10^{-6} , 10^{-7} ; series 2: 10^{-5} , 10^{-6} , 10^{-7} ; series 3: 10^{-6} , 10^{-6} , 10^{-7} ; series 4: 10^{-5} , 10^{-6} , 10^{-7} ; and series 5: 10^{-5} , 10^{-5} , 10^{-6} .

Discussion

Our study shows the value of RNA detection by NASBA for the monitoring of *C trachomatis* infections after antibiotic treatment. One week after the start of such treatment, *C trachomatis* RNA could be detected in only two cervical smears, and after two weeks of treatment, no *C trachomatis* RNA was detectable (fig 2A). Also, in those cases with cell culture proven *C trachomatis* infections, only one case showed *C trachomatis* RNA one week after antibiotic treatment, while cell cultures were negative up to four weeks after treatment. These cell culture results are in complete agreement with those obtained in other studies.^{5 6 9-11} Some groups^{4 6} reported culture positive results after antibiotic treatment. However, these data appeared to be derived from patients with a history of continued unprotected sexual intercourse with an untreated *C trachomatis* infected partner.

In contrast to *C trachomatis* RNA, *C trachomatis* DNA could be detected in many of the cervical smears one week after antibiotic treatment (Fisher's exact test, $p < 0.00001$; two tailed, OR = 46 (95% CI, 9.8 to 216.1)). In all patients showing consistent *C trachomatis* DNA results, *C trachomatis* DNA disappeared from the cervical smears two to three weeks after antibiotic treatment (the four patients with reappearing *C trachomatis* DNA will be discussed later). These results are in agreement with other studies, in which *C trachomatis* DNA could also be detected up to two^{6 11} or three weeks^{6 9} after antibiotic treatment using PCR. These data demonstrate that the presence of *C trachomatis* DNA after antibiotic treatment

reflects excretion of non-viable organisms because cell culture was negative in these studies. In all cases where no *C trachomatis* RNA could be detected by NASBA, cell culture results were always negative, despite the presence of *C trachomatis* DNA. Therefore, RNA detection by NASBA is likely to be more clinically relevant than testing for DNA by PCR after antibiotic treatment. Furthermore, RNA detection is not an absolute marker for *C trachomatis* viability because *C trachomatis* RNA was detected one week after treatment, whereas cell culture was negative. However, these few discrepancies between cell culture and NASBA results could also be explained by the relative insensitivity of cell culture in the detection of *C trachomatis* infections in clinical specimens (such as inefficient culture because of low titre infections, effects of transport, or inhibition). Moreover, PCR and NASBA were 0–10 and 10–100 times more sensitive, respectively, compared with cell culture using dilution series of clinical isolates. When taking into account the proportion of the original sample used for analysis (40 times more for cell culture) the higher sensitivity of the amplification assays compared with cell culture becomes even more evident.

The main advantages of RNA detection by NASBA, as compared with cell culture for the identification of viable microorganisms, include its high sensitivity and reliability resulting from the internal standard used. Furthermore, the results can be obtained within one working day. In addition to the monitoring of antibiotic treatment, RNA detection could also be a good alternative for studying the activity of new antibiotics in vitro and could be used to compare the activities of different antibiotics in vivo, using both cervical smears and urine specimens.

Before antibiotic treatment, the urine specimens of all patients were clearly positive for both *C trachomatis* DNA and RNA (fig 2B). In such samples, a prominent decrease in *C trachomatis* RNA v DNA was found one week after antibiotic treatment (one of 15 v four of 15; $p = 0.3295$; two tailed, not significant (NS)), and no *C trachomatis* DNA or RNA were found two weeks or more after treatment. In one urine specimen in which *C trachomatis* RNA could be detected after antibiotic treatment, *C trachomatis* DNA and RNA were also detected in the corresponding cervical smear. No difference in *C trachomatis* RNA clearance was found between urine specimens and cervical smears ($p = 1.00001$; two tailed, NS). In contrast, in most cases with a *C trachomatis* positive cervical smear one week after treatment, the corresponding urine specimen appeared to be *C trachomatis* DNA negative ($p = 0.00206$; two tailed, OR = 11 (95% CI, 2.3 to 52.6)). This difference in *C trachomatis* DNA detection might be explained by the fact that residual *C trachomatis* organisms present in the urinary tract were eradicated through frequent urination, whereas the cervical mucosa may have retained these residual organisms for a longer time. The difference in eradication time could also reflect a difference

in antibiotic levels achieved in the cervix versus the urethra, because doxycycline is found in the urine at high concentrations. Two weeks after antibiotic treatment, *C trachomatis* RNA was no longer detected in both cervical smears and urine specimens, and *C trachomatis* DNA was not detected in urine specimens. This quick eradication of *C trachomatis* DNA and RNA in the urinary tract makes urine potentially suitable for use as a test of cure using NASBA or PCR. The use of urine will facilitate sample collection greatly because of its non-invasiveness, in contrast to the use of cervical smears. As described in different studies using both PCR and LCR for the detection of *C trachomatis*¹³⁻¹⁶ urine specimens could be used reliably instead of cervical smears.

Interestingly, the reappearance of *C trachomatis* DNA in cervical smears after a 1-2 week *C trachomatis* DNA free interval was seen in four cases (table 1). However, these cases were only weakly positive after Southern hybridisation. These fluctuating results might be caused by sampling differences in the specimens. Alternatively, the *C trachomatis* DNA reappearance might reflect reinfection with *C trachomatis* or reactivation upon withdrawal of antibiotics. However, this is unlikely because no *C trachomatis* RNA could be detected in these specimens (the NASBA internal standard was visible, indicating that sample preparation was adequate and that inhibition of amplification had not occurred). Moreover, from two of these four patients with reappearing *C trachomatis* DNA (patients 1 and 3, table 1), cervical smears were again negative for both *C trachomatis* DNA and RNA one week later (weeks 5 and 4, respectively). This suggests strongly that the *C trachomatis* DNA reappearance was not related to reinfection or to reactivated chlamydia infection, but originated from residual non-viable material. Therefore, our study did not find any evidence for in vivo persistent *C trachomatis* infections. In contrast, these persistent *C trachomatis* infections have been described in vitro, characterised by the presence of enlarged, atypical chlamydia forms that are non-infectious but retain viability. Suppression by antibiotics, cytokines, and nutrient depletion are responsible for these persistent forms in cell culture.^{7 8}

In conclusion, our study shows that RNA detection by NASBA can be used successfully for the monitoring of *C trachomatis* infections after treatment with antibiotics. Furthermore, it might be possible to use urine specimens as a test of cure using either NASBA or PCR, because neither *C trachomatis* DNA or RNA could be detected two weeks after antibiotic treatment.

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