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Comparison of Characteristics of Qβ Replicase-Amplified Assay with Competitive PCR Assay for *Chlamydia trachomatis*

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In order to study infections due to *Chlamydia trachomatis*, we have compared semiquantitative PCR and Qβ replicase-amplified assays for detection of this organism. The PCR assay was directed against the *C. trachomatis* 16S rRNA gene. Quantitation was accomplished by adding known amounts of a plasmid containing a truncated segment of the 16S rRNA gene target to chlamydia-containing samples and then amplifying with a common primer set. The Qβ replicase assay consisted of reversible target capture of *C. trachomatis* 16S rRNA, which was followed by amplification of an RNA detector probe in the presence of the enzyme Qβ replicase. In a clinical matrix, the lower limit of detection of both the PCR and Qβ replicase assays was five elementary bodies. The Qβ replicase and PCR assays were quantitative over 10,000- and 1,000-fold ranges of organisms, respectively. Analysis of the effects of endocervical matrix on amplification was accomplished by examining 94 endocervical specimens by each technique. Both assays detected five of six culture-confirmed specimens as well as three culture-negative specimens. PCR inhibitors were detected in 13 specimens. The Qβ replicase assay, in contrast, showed no evidence of sample inhibition. The Qβ replicase and PCR assays should allow quantitative investigation of infections due to *C. trachomatis*. In addition, because it targets highly labile RNA, the Qβ replicase assay may facilitate investigations into the role of active persisting infection in culture-negative inflammatory conditions.

Chlamydia trachomatis is one of the most common causes of sexually transmitted disease in the United States. The clinical manifestations of *C. trachomatis* infection are diverse and may include urethritis, cervicitis, endometriosis, salpingitis, and perihepatitis (19). Chlamydia infections are often asymptomatic or nonspecific in their initial clinical presentations. This, combined with difficulties in isolation, propagation, and identification of the organisms, has complicated analysis of the pathologies associated specifically with chlamydial infection (2).

Quantitative amplified nucleic acid detection systems should facilitate analysis of the pathophysiology due to *C. trachomatis* especially with regard to monitoring persistence of the organisms following incomplete antibiotic treatment, which can lead to recurrent infection. We previously have used RNase protection and PCR assays to detect *C. trachomatis* in endocervical specimens taken from women presenting with a variety of symptomatologies (1). On the basis of results obtained from the RNase protection assay, we noted significant differences in the apparent levels of *C. trachomatis* 16S rRNA from patient to patient. The RNase protection assay indicated the presence of levels of 16S rRNA as high as 10^9 copies, equivalent to 10^6 elementary bodies (EBs), per 100 μl of sample fluid from culture-negative, PCR-positive samples. Due to the lability of 16S rRNA, its persistently high levels in these patient specimens would seem to indicate active infection rather than the presence of dead organisms. The limited sensitivity of the RNase protection assay, however, restricted our ability to analyze culture-negative samples containing chlamydia-specific nucleic acid.

We therefore have developed a semiquantitative competitive PCR assay for the 16S rRNA gene of *C. trachomatis* and compared it with an assay based on reversible target capture of *C. trachomatis* 16S rRNA followed by Qβ replicase amplification of a replicatable RNA detector molecule (9, 12–17). The PCR and Qβ assays were compared with respect to the number of molecules of target which could be detected, the range of target concentration over which the assays were quantitative, and the effects of clinical matrix on assay results. Both assays have potential use for quantitative analysis of *C. trachomatis* infection and may be used to investigate potential sequelae by these organisms.

MATERIALS AND METHODS

Patient specimens and cell culture. Coded samples were submitted to our laboratory from the University of Massachusetts Medical Center, as previously described (1). The patients were from a family medicine clinic or a gynecology clinic associated with the hospital or were patients admitted to the University of Massachusetts Hospital. The samples were obtained prior to the administration of antibiotics from patients with symptoms consistent with a sexually transmitted disease. Female endocervical samples were obtained by first removing mucus from the cervix with a swab which was then discarded. A calcium alginate swab was inserted into the endocervical canal and rotated gently 180°. Swabs were placed immediately into 1 ml of 0.2 M sucrose-phosphate medium containing gentamicin (5 μg/ml), vancomycin (12.5 μg/ml), and nystatin (12.5 μg/ml) and stored at −70°C. Specimens were thawed, and 100-μl aliquots were inoculated in duplicate onto cultures of HeLa-229 and McCoy cells seeded on coverslips (12 mm) in glass vials (Viromed Inc., Minmetonka, Minn.). The cell cultures were pretreated with DEAE-dextran (30 μg/ml) in phosphate-buffered saline for 30 min at 37°C prior to inoculation. The cultures were centrifuged at 1,000 × *g* at room temperature for 1 h, after which 1 ml of Eagle's minimal essential medium containing 10% fetal calf serum was added and the cultures were incubated at 37°C for 48 h. After incubation, the media were removed and the cells were fixed in methanol. Coverslips were removed, stained with either a genus- or a species-specific fluorescein-conjugated monoclonal antibody (Kallestadt Inc., Chaska, Minn.), and read for chlamydial inclusions. Cultures exhibiting one or more fluorescent inclusions were considered positive for the presence of *C. trachomatis.*

Growth of *C. trachomatis*. *C. trachomatis* EBs were prepared from infected McCoy cell cultures as previously described (1). Chlamydiae were harvested when the monolayers had 15 to 30 inclusions per high-power microscopic field and approximately 30 to 50% of the cell monolayer appeared to be infected. EBs were purified by a series of centrifugations, DNase treatment, and final banding

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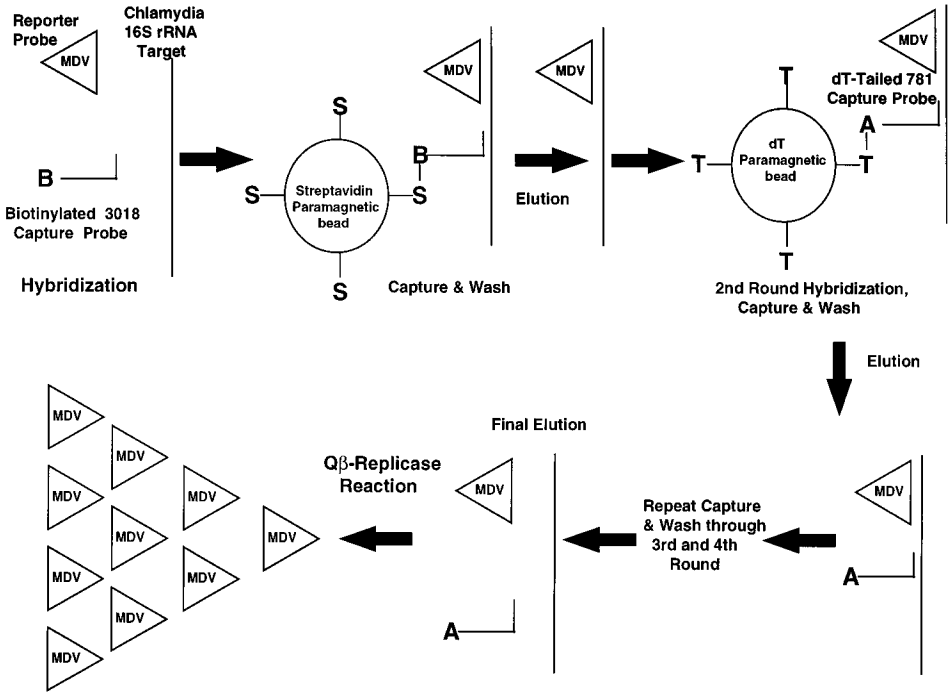

FIG. 1. Schematic representation of the reversible target capture and Qβ replicase-amplified assay.

on a Renografin-76 (E. R. Squibb and Sons, Princeton, N.J.) gradient. The concentration of EBs was determined by direct counts of preparations stained with a fluorescent antibody (Kallestadt).

Extraction and quantitation of chlamydia nucleic acid. Chlamydia rRNA was purified by a modification of the procedure described by Barns et al. (3). The nucleic acid was combined with cesium trifluoroacetate (Pharmacia Inc., Piscataway, N.J.), and the high-molecular-weight RNA was pelleted by equilibrium density centrifugation (>4 h at 150,000 × *g* at 4°C) in a TLA 100.3 rotor with a Beckman TL100 ultracentrifuge. Pellets were suspended in 200 μl of TMK buffer (10 mM Tris-HCl [pH 7.4], 5 mM $MgCl_2$, 30 mM KCl), precipitated with sodium acetate and ethanol, dried, and dissolved in RNase-free water. The RNA was quantitated by spectrophotometry.

Oligonucleotides and nucleic acid probes. The PCR primers PfRNA (5′-GAAGGCGGTTAATACCCGCTG-3′) and PrRNA (5′-GATGGGGTTGAGACCATCC-3′), used to amplify the *C. trachomatis* 16S rRNA gene, have been previously characterized (1). PCR amplification was confirmed by hybridization to either a ^{32}P-labeled oligonucleotide probe, designated P_{D1} (5′-CTTTTCTAATTTATACCTGAC-3′), which detected both the 398-bp *C. trachomatis* amplicon and the 231-bp amplicon generated by amplification of the control plasmid pG231 or a similarly labeled detector probe, designated P_{D2} (5′-GGCTAGAAACTGTTGATTGAATG-3′), which detected only the 398-bp *C. trachomatis* amplicon.

The reversible target capture and Qβ replicase amplification procedure was carried out with two capture probes. The 3018 probe (5′-CCTTTAACGTTACTCGGATGCCCAAA-3′) contained an aminopropyl-modified cytosine at the 5′ end. Biotin was attached to the 5′-amino-modified base, using a FluoReporter biotin labeling kit (Molecular Probes Inc., Eugene, Ore.). The 781 probe (5′-CTTTAACGTTACTCGGATGCCCAAATATCGCCACAT-3′) was modified by addition of a tail of approximately 150 dA residues, as described by Morrissey et al. (14, 15).

The replicatable detector probe was a recombinant midivariant (MDV) RNA (12) containing the sequence (5′AGGCCTTTACCCCACCAACTAGCTGATATCACATA-3′) complementary to chlamydia 16S rRNA target. This sequence plus flanking 10-nucleotide spacer elements were inserted between nucleotides 63 and 64 of the plus-strand RNA (6a). The vector plasmid was linearized by digestion with *Sma*I, and the detector probe was prepared by transcription of a recombinant vector with T7 RNA polymerase (Megascript kit).

Construction of an internal control plasmid for PCR assays. An internal control plasmid for the PCR assay was constructed and used to detect sample inhibition of PCR. The 398-bp amplicon resulting from amplification of the *C. trachomatis* 16S rRNA gene was isolated and digested with *Pvu*II and *Pml*I. The two end fragments (67 and 174 bp) containing the primer sites were purified, ligated, and cloned into a T/A vector (Invitrogen, San Diego, Calif.). A plasmid, designated pG231, containing an internally deleted 231-bp fragment of the *C. trachomatis* 16S rRNA gene amplicon was isolated and used as an internal standard in all PCR assays.

PCR assay. The PCR assay conditions for detection of the *C. trachomatis* 16S rRNA gene have been previously described (1). Clinical samples or EBs were prepared similarly for PCR and Qβ replicase assays. Briefly, a 500-μl portion of sample was pelleted by centrifugation in a microcentrifuge for 10 min at 13,000 × *g*. The supernatant was removed and the pellet was suspended in 200 μl of a 1% Nonidet P-40 (NP-40) solution. A 100-μl aliquot of each sample was transferred to a new tube and set aside for the Qβ replicase assay. A second 100-μl aliquot of each sample was transferred to a new tube and heated at 95°C for 10 min in a heat block. For PCR, 25 μl of sample was amplified in the presence of 1× amplification buffer (Perkin-Elmer Cetus, Norwalk, Conn.)–2.5 mM $MgCl_2$–2.5 U of Amplitaq polymerase (Perkin-Elmer Cetus) in a total volume of 100 μl. All PCR assays were carried out under the recommended containment conditions (11). The 398-bp amplicon generated with primers PfRNA and PrRNA was detected either on ethidium bromide-stained agarose gels following electrophoresis or by Southern blot hybridization with a radiolabeled oligonucleotide probe specific for the amplicons.

Paramagnetic particles. Paramagnetic particles were obtained from Advanced Magnetics Inc. (Cambridge, Mass.). The particles were derivatized with either oligo(dT) or streptavidin as previously described (9, 14, 15, 17).

Qβ replicase assay. The Qβ replicase assay is shown in schematic form in Fig. 1. The assays were run in a 96-well format, using sample racks and magnetic separators as previously described (13, 17). Portions of the samples prepared in NP-40 for the PCR assay were used for the Qβ replicase assay. A 100-μl portion of either purified 16S RNA, EBs, or an endocervical sample was combined with 400 μl of sample processing buffer (8.0 M GuHCl, 0.6% Sarkosyl, 100 mM Tris-HCl [pH 7.8], 20 mM EDTA), and the mixture was vortexed for 30 s. A 100-μl portion was removed and analyzed for the presence of *C. trachomatis* in a combined reversible target capture Qβ replicase-amplified assay. The biotinylated 3018 capture probe and *C. trachomatis*-specific detector probe were diluted in probe dilution buffer (100 mM Tris-HCl [pH 7.8], 20 mM EDTA) to a final concentration of 300 ng/ml each. Hybridization reactions (total volume, 100 μl) were prepared by combining 66.6 μl of sample with 33.3 μl of the probe mixture, yielding a final concentration of 100 ng/ml for both the capture and detector probes. The probes were hybridized with the target nucleic acid for 30 min at 37°C. Following hybridization, 100 μl of a suspension of 0.04% streptavidin-coated paramagnetic particles in bead buffer (100 mM Tris-HCl [pH 7.8], 20 mM EDTA, 4% bovine serum albumin, 0.5% Sarkosyl, 0.01% antifoam) was added, and the reaction was incubated further at 37°C for 5 min. The particles were collected on the sides of the reaction tubes by exposure to a magnet for 2 min. The particle-bound ternary complexes were washed with 200 μl of low-salt buffer (100 mM Tris-HCl [pH 8.1], 20 mM EDTA, 25 mM NaCl, 0.2% Sarkosyl, 0.05% bovine serum albumin, 0.05% Bronopol). Following vortexing, the particles were

collected as before and the wash was repeated. The binary target-detector probe complex was released from the particles by addition of 100 μl of 3 M GuSCN release buffer (240 mM Tris-HCl [pH 7.8], 60 mM EDTA, 3 M GuSCN, 0.6% Sarkosyl). The biotinylated 3018 probe was left bound to the first set of particles. After a 5-min incubation in release buffer, the particles were again collected, and the supernatant containing the binary target-detector probe complex was transferred to a fresh tube.

A 50-μl portion of a 300-ng/ml solution of dA-tailed 781 capture probe in probe dilution buffer was added, and the tubes were incubated for 30 min at 37°C. Following hybridization of the dA-tailed 781 probe to the binary target-detector probe complex, 250 μl of 0.05% oligo(dT) particles in bead blocking buffer was added, and the ternary capture probe-target-detector probe complexes were captured onto the particles for 5 min at 37°C. The particles were separated as before and washed three times with 200 μl of high-salt wash buffer (100 mM Tris-HCl [pH 8.1], 300 mM NaCl, 0.5% Sarkosyl, 0.5% bovine serum albumin, 0.5% Bronopol). The ternary complexes were eluted from the particles by the addition of 100 μl of low-salt buffer. The particles were collected as before, and the supernatant containing the ternary complexes was transferred to a fresh tube.

A 100-μl portion of a fresh 0.12% suspension of oligo(dT) particles and 200 μl of 8 M GuHCl dilution buffer (100 mM Tris-HCl [pH 7.8], 20 mM EDTA, 8 M GuHCl) were added, and the ternary complexes were captured and washed as described above. The elution and capture were repeated through a fourth round. Following completion of the washing steps, the target-detector probe complex was released by addition of 150 μl of preamplification release buffer (50 mM Tris-HCl [pH 8.0], 1 mM EDTA, 0.5% NP-40), and the eluted material was transferred to a fresh tube. A 100-μl portion of the released hybrid complex was combined with an equal volume of Qβ replicase buffer (220 mM Tris-HCl [pH 7.8], 40 mM $MgCl_2$, 1.2 mM [each] GTP, ATP, CTP, and UTP, 2.0 μg of propidium iodide per ml, 25% glycerol, 110 μg of Qβ replicase per ml) in a closed tube. The reactions were mixed and placed in a custom-built recording fluorimeter at 37°C (6a). The reactions were illuminated at 510 nm, and fluorescence was measured at 615 nm through the bottom of the tube, using a split fiber-optic bundle. Fluorescence was measured in all 96 reactions simultaneously at 40-s intervals for 45 min. Fluorescence increased in those reactions containing replicatable detector probe. The time interval required to produce sufficient RNA such that its fluorescence was detectable above the basal level is defined as the response time and correlates to the presence of approximately 3×10^{11} molecules of the detector probe (6a). The response time is inversely proportional to the log of the number of detector molecules which initiate the amplification reaction (12, 14, 17). Thus, a short response time would indicate the presence of a large number of detector molecules and a correspondingly large number of target molecules in the initial sample. Positive and negative controls for each assay consisted of samples containing known numbers of either EBs, 16S rRNA molecules, or Tris-EDTA buffer.

Effect of endocervical matrix on assay performance. In order to examine the effect of clinical matrix on PCR, processed endocervical specimens were combined with 500 molecules of the pG231 plasmid and subjected to 40 cycles of amplification. Samples were analyzed in duplicate. In order to minimize differences in PCR reaction conditions, all reagents were added from a single master mixture. Samples and controls were also amplified and analyzed simultaneously, using a single thermocycler and agarose gel. The appearance of the 231-bp amplicon resulting from amplification of the pG231 plasmid was used to determine the presence or absence of PCR-inhibitory substances. Specimens in which PCR inhibitors were detected were extracted with phenol and chloroform (Insta-Prep; 5′-3′ Inc., Boulder, Colo.) and reamplified. For samples in which amplification occurred, 10 μl of PCR product was analyzed by agarose gel electrophoresis, stained with ethidium bromide, photographed, and quantitated either by densitometric scanning (Masterscan [CSPI, Billerica, Mass.] or NIH Image Software [National Institutes of Health, Bethesda, Md.]) or Phosphorimager analysis of Southern blots (Molecular Devices, Sunnyvale, Calif.), using the method described by Siebert and Larrick (18).

Samples to be analyzed by the Qβ replicase assay were prepared and tested as described above. An amplified dose response of quantitated, serially diluted, purified 16S rRNA was performed in each experiment as a control. Each concentration of the control dose response was assayed in quadruplicate. The control dose response was used to establish the lower limit of detection and to set a cutoff for the clinical Qβ replicase assay. Clinical samples were assayed by Qβ replicase assay in duplicate.

RESULTS

Optimization and characterization of a competitive PCR assay for *C. trachomatis*. The competitive PCR was optimized initially by determining the concentration of the pG231 plasmid which resulted in the greatest molecular sensitivity. We determined that 500 molecules of plasmid pG231 yielded optimal sensitivity. As shown in Fig. 2A, when 500 molecules of pG231 were coamplified with decreasing amounts of *C. trachomatis* EBs in Tris-EDTA buffer, the sensitivity by ethidium bromide-stained gel was five organisms. Hybridization of Southern blots with radiolabeled probe P_{D1} (Fig. 2B) or P_{D2} (Fig. 2C) did not significantly increase the sensitivity beyond five organisms.

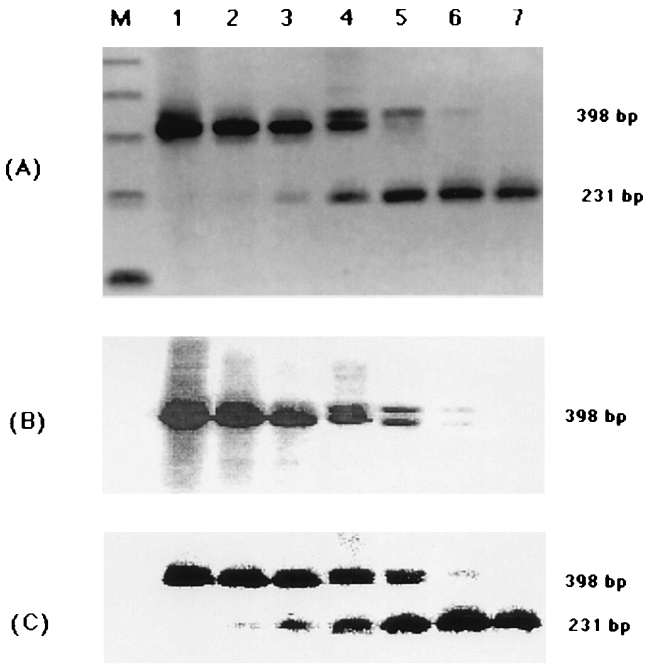

FIG. 2. Coamplification of *C. trachomatis* EBs and plasmid pG231, illustrating optimal conditions. (A) Lanes 1 to 7 show coamplification of decreasing amounts of *C. trachomatis* EBs with 500 molecules of the plasmid pG231 DNA analyzed by ethidium bromide-stained agarose gel electrophoresis: lane 1, 5×10^5 EBs; lane 2, 5×10^4 EBs; lane 3, 5×10^3 EBs; lane 4, 5×10^2 EBs; lane 5, 5×10^1 EBs; lane 6, 5 EBs; and lane 7, pG231 only. (B) Southern hybridization of the agarose gel in panel A with the P_{D1} detector probe. (C) Rehybridization of the Southern blot in panel B with the P_{D2} detector probe. Lane M is a 123-bp DNA ladder used as a molecular size marker.

For characterization of assay performance on unknown numbers of organisms in a simulated clinical matrix, EBs were combined with bovine cervical mucus (1) and 0.2 M sucrose-phosphate buffer and processed as described above. Each sample was amplified by PCR as described, using an input of 500 molecules of the pG231 plasmid. Serial dilutions of a titered stock of *C. trachomatis* EBs in buffer were combined with 500 molecules of the pG231 plasmid and were amplified directly as controls. The amounts of the *C. trachomatis* and pG231 amplicons in the control reactions and the experimental samples were determined following gel electrophoresis and analysis by densitometry (18).

Figure 3 illustrates the quantitative results. The assay was linear over a range of 5 to 5,000 EBs. The limit of detection for the PCR assay was five EBs. Since there are two copies of the 16S rRNA gene per EB (23), this represents 10 molecules of target.

Characterization of the Qβ replicase assay for *C. trachomatis*. Serial dilutions of known amounts of 16S rRNA were used to establish the characteristics of the Qβ replicase assay. Figure 4 illustrates the relationship between the number of molecules of 16S rRNA and the response time in the Qβ amplification reaction. The relationship between the response time and the number of molecules of target in the initial sample can be summarized as follows: $N = N_0\, 2^{t/d}$, where N equals the number of molecules present at arbitrary time, t. For these calculations, t is taken as the response time, which in

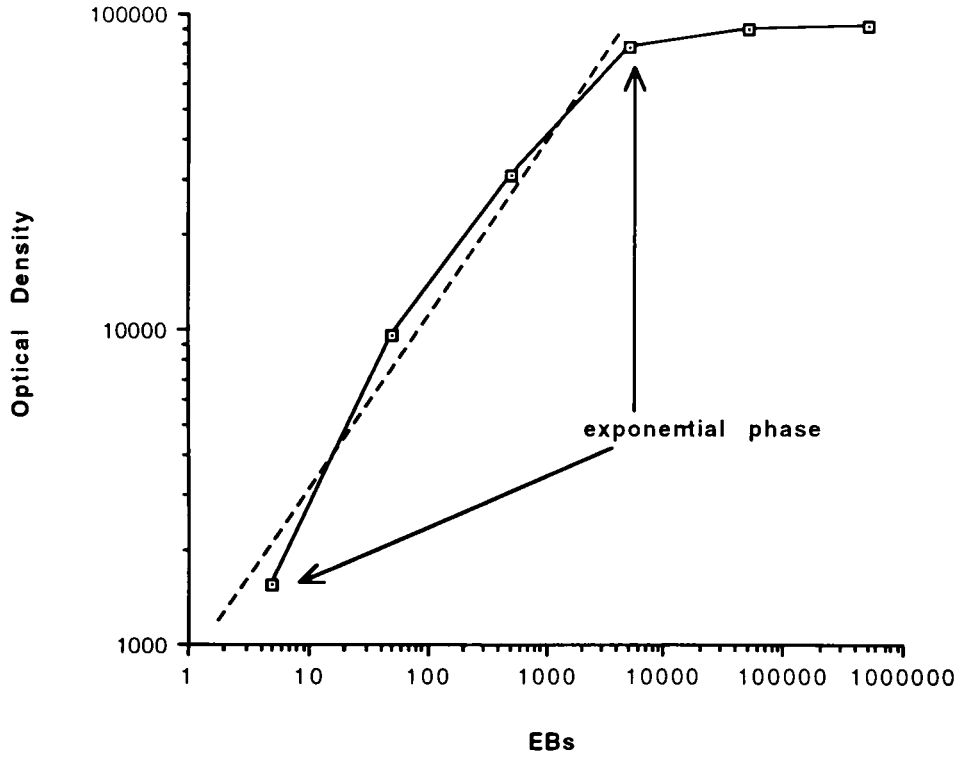

FIG. 3. Quantitation of *C. trachomatis* EBs by PCR. The products of the PCR reaction were separated on an agarose gel, stained with ethidium bromide, and analyzed by densitometry. The optical density of the gel bands as determined by densitometry were plotted against the number of EBs assayed.

this system corresponds to the time it takes the reaction to reach the end of the exponential phase of amplification, which is a constant 3.0×10^{11} molecules. N_0 represents the number of molecules in the original sample, and d represents the doubling time, which is a constant under the given reaction conditions and a given replicating detector probe. Therefore, knowing the response time, t, the number of molecules of target in the original sample can be determined (7, 13).

An inverse semilogarithmic relationship between target concentration and response time was maintained over the 10,000-fold range of target concentration tested. The concentration of rRNA in either a preparation of 16S rRNA, EBs, or a clinical sample was calculated according to the method described above. The assay had a lower limit of detection of 1,000 molecules of 16S rRNA (Fig. 4).

For characterization of the Qβ replicase assay's ability to detect EBs in a simulated clinical matrix, known numbers of EBs were combined with bovine cervical mucus and 0.2 M sucrose-phosphate buffer and processed as described above. The results were compared with those obtained from amplification of quantitated, serially diluted *C. trachomatis* 16S rRNA. The comparison of the response times of the EB-containing samples with the concentration of 16S rRNA is shown in Fig. 4. The assay again showed an inverse semilogarithmic relationship between response time and the number of EBs in each sample over the range of 5 to 50,000 EBs tested. The assay was quantitative down to the level of a single EB.

Effect of endocervical matrix on PCR and Qβ replicase assay performance. Analysis of 94 clinical specimens was performed to examine the effects of clinical matrix on each assay. For PCR analysis of clinical samples, endocervical specimens were processed as described above, combined with 500 molecules of the pG231 plasmid, and subjected to 40 cycles of amplification. Amplification products from PCR assays were analyzed by Southern blot. An equal portion of the specimen was removed and combined with GuHCl sample processing buffer, and the mixture was vortexed for 30 s and assayed in the Qβ replicase system.

For these experiments, 94 endocervical specimens were examined for inhibitory effects using both assays in parallel. Six specimens were positive by culture. Although one of these specimens gave negative results in both the PCR and Qβ replicase assays, neither result was due to sample inhibition. The remaining five culture-positive specimens were also positive by Qβ replicase and PCR assays. In addition, three specimens negative by culture were positive by both PCR and Qβ replicase assays. These samples may represent false-negative culture results. Thirteen of the 94 samples tested (13.8%) showed evidence of PCR inhibition following NP-40 lysis and direct amplification. Following further purification by extraction with phenol-chloroform and ethanol precipitation, however, eight of the inhibited specimens could be amplified and were negative for *C. trachomatis*. Five samples showing complete inhibition upon initial amplification of the NP-40 lysates were not amplifiable even after phenol extraction. A known number of EBs spiked into PCR-inhibited samples gave the expected level of target as assessed by the Qβ replicase assay and were therefore not inhibitory to the assay. The PCR, Qβ replicase, and culture assays were in perfect concordance for the remaining 72 *C. trachomatis*-negative specimens, and none of the assays showed any signs of inhibition.

The analysis of the *C. trachomatis*-positive specimens is shown in Table 1. On the basis of the previously reported 500 to 1,000 molecules of rRNA per copy of the 16S rRNA gene (1), the quantitative estimates of the number of targets present

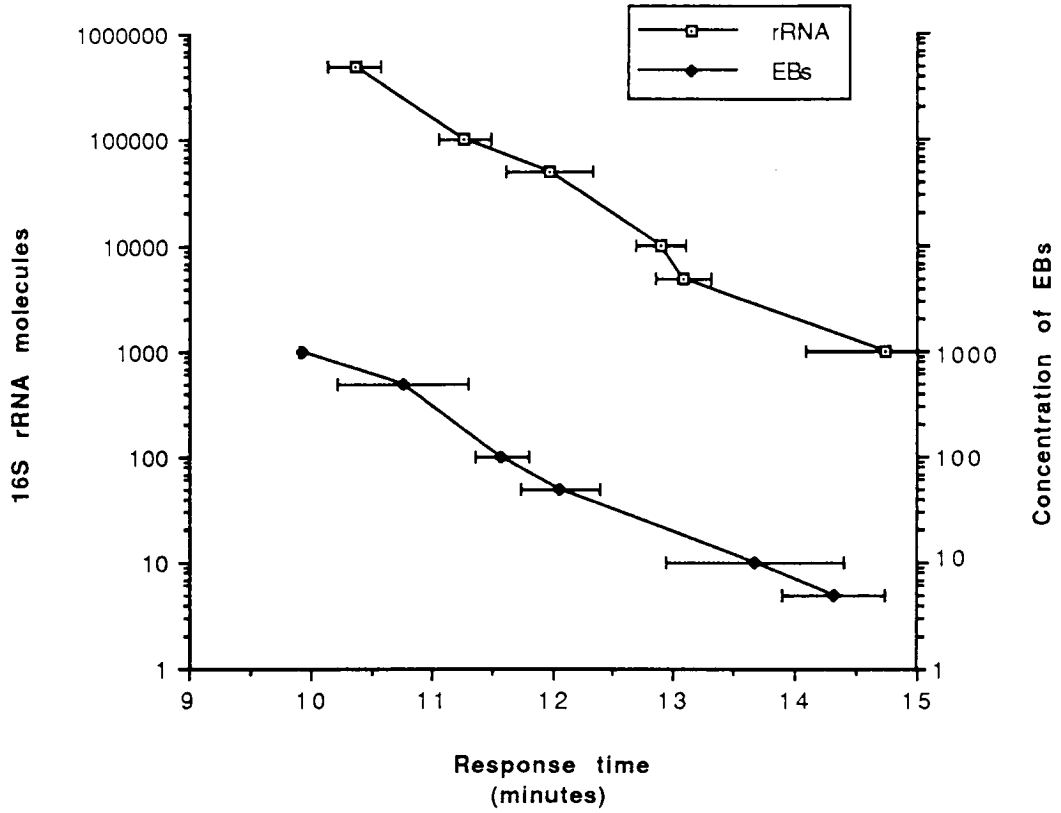

FIG. 4. Response times of Qβ replicase amplification reactions for purified rRNA and EBs. Each assay point represents the average of four replicate values. The error bars indicate the standard deviations of the response times. All samples containing 1,000 or more molecules of *C. trachomatis* 16S rRNA gave response times in the assay. Samples containing fewer than 1,000 molecules were variable in yielding a positive result and were considered below the limit of detection of the assay. Samples without target were uniformly negative.

in the endocervical samples by PCR and Qβ replicase assays were in general agreement.

DISCUSSION

We have compared two semiquantitative amplification assays for *C. trachomatis*. The region of the 16S rRNA gene detected by the PCR assay was designed such that it did not overlap the region homologous with the Qβ replicase assay probes to avoid assay interference. We chose to use the 16S rRNA gene as a target of the PCR assay rather than the cryptic plasmid because of our previous observation that specimens from patients who have previously undergone initial antibiotic treatment for a chlamydia infection that is followed by later onset of pelvic inflammatory disease can contain chlamydia-specific rDNA and high levels of chlamydia-specific 16S rRNA yet lack the cryptic plasmid (1). Although PCR assays directed against the cryptic *C. trachomatis* plasmid have been reported to have molecular sensitivities of a single EB, a commercially available assay directed against the plasmid target has shown variable clinical sensitivity (1, 4, 10). In addition, the difference between one and five EBs is not statistically significant when the assay endpoints are titrated, given errors in pipetting and dilution. In practice, we have seen no difference in the molecular sensitivity of PCR assays targeted against either the cryptic plasmid or the 16S rRNA, and in order for the assay performance to be comparable on all specimens, we used the 16S rRNA gene as a target.

TABLE 1. Analysis of endocervical specimens positive for *C. trachomatis* by culture, Qβ replicase, or PCR assays

Sample no.	Culture result[a]	Molecules of target[b]	
		PCR	Qβ replicase assay
38	−	1.07E + 3[c]	2.05E + 5
45	+	7.06E + 2	5.86E + 4
46	+	1.88E + 3	1.01E + 5
47	+	5.15E + 3	6.27E + 5
49	+	3.05E + 3	3.01E + 5
50	+	1.90E + 3	2.33E + 4
54	−	6.60E + 2	2.83E + 4
74	−	4.07E + 2	1.43E + 4

[a] −, negative; +, positive.
[b] Average of two determinations.
[c] 1.07E + 3 = 1.07×10^3.

We have shown here that the Qβ replicase assay is capable of easily quantitating a 10,000-fold range of target input. Lizardi et al. (12) have used a Qβ assay to detect human immunodeficiency virus RNA and have demonstrated that the assay was linear over a 10^9-fold range of target concentration, making the Qβ replicase assay extremely adaptable to the varying concentrations of organisms which might be found in clinical samples (12). The PCR assay was quantitative over a 1,000-fold range of concentrations of *C. trachomatis* EBs with an upper limit to these assay conditions of 5.0×10^3 organisms. However, this upper limit may not be adequate for all patient samples. We have previously shown that patients with infections due to *C. trachomatis* can have rRNA concentrations as

great as 10^9 molecules per 100 μl of sample assayed (1). This is equivalent to 2.0×10^6 EBs and is beyond the quantitative range of the PCR assay described here. In order to achieve a different range of quantitation, the PCR assay would have to undergo reoptimization in terms of fewer amplification cycles to quantitate higher levels of organisms. Effectively, this would not increase the quantitative ability of the assay but would, rather, shift the 1,000-fold range of quantitation.

We detected PCR-inhibitory effects in 13.8% of the clinical specimens. The inhibitory effects ranged from partial to complete inhibition of the amplification. The majority of the patients surveyed here had specimens free of excessive blood and discharge. Sample inhibition of PCR in endocervical specimens has been reported in other studies and may be a significant problem (4). In contrast, sample-inhibitory effects were not observed in the Qβ replicase assay. This was not surprising since the actual amplification is preceded by a purification step, reversible target capture, which has been reported to decrease interfering substances by 10^{12}- to 10^{16}-fold following four rounds of cycling (10, 12, 13, 17).

Sensitive methods for the analysis of *C. trachomatis* infections may be necessary for effective management of potentially serious disease. One of the most serious complications of chlamydia infection is the progression to inflammatory sequelae that leads to blindness or infertility. While the occurrence of persistent *C. trachomatis* infections is not clearly established, several studies indicate that these infections may occur and contribute to the pathogenesis of the organism, especially with regard to sequelae (6, 8, 20–22).

The mechanisms by which the infections persist, however, are unknown. Nucleic acid studies have shown that DNA and RNA can persist well beyond the period of detection by culture or direct fluorescent-antibody staining (6, 8). However, differentiating live from dead organisms is problematic in analyzing persistent infections. PCR, as performed on DNA, cannot differentiate live from dead organisms. In contrast, the detection of RNA is strongly suggestive of the persistence of live chlamydiae. Unlike DNA, RNA is subject to rapid turnover in infected cells. Holland et al. (8) have suggested that there may be an advantage to following rRNA by hybridization in comparison to DNA detection by PCR for the purpose of monitoring active infections due to *C. trachomatis.*

The Qβ replicase assay and competitive PCR should be extremely useful in monitoring active infections due to *C. trachomatis* in a more sensitive manner than can be accomplished with tissue culture or direct fluorescent-antibody staining. These assays may also facilitate investigation of the role of persistent infection in culture-negative inflammatory conditions.

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