SCIENTIFIC REPORT

Comparison of an rRNA-based and DNA-based nucleic acid amplification test for the detection of *Chlamydia trachomatis* in trachoma

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Background/Aim: The World Health Organisation (WHO) hopes to achieve global elimination of trachoma, still the leading cause of preventable blindness worldwide, in part through mass antibiotic treatment. DNA-based nucleic acid amplification tests (NAATs) are currently used to evaluate the success of treatment programmes by measuring the prevalence of *C trachomatis* infection. Some believe that newer ribosomal RNA (rRNA)-based tests may be much more sensitive since bacterial rRNA is present in amounts up to 10 000 times that of genomic DNA. Others believe that rRNA-based tests are instead less sensitive but more specific, due to the presence of dead or subviable organisms that the test may not detect. This study compares an rRNA-based test to a DNA-based test for the detection of ocular *C trachomatis* infection in children living in trachoma-endemic villages.

Methods: An rRNA-based amplification test and DNA-based polymerase chain reaction (PCR) were performed on swab specimens taken from the right upper tarsal conjunctiva of 56 children aged 0–10 years living in two villages in Amhara, Ethiopia.

Results: The rRNA-based test detected ocular *C trachomatis* infection in 35 (63%) subjects compared with 22 (39%) detected by PCR (McNemar's test, $p=0.0002$). The rRNA-based test gave positive results for all subjects that were positive by PCR, and also detected infection in 13 (23%) additional subjects.

Conclusion: The rRNA-based test appears to have significantly greater sensitivity than PCR for the detection of ocular chlamydial infection in children in trachoma-endemic villages. Using the rRNA-based test, we may be able to detect infection that was previously missed with PCR. Past studies using DNA-based tests to assess prevalence of infectious trachoma following antibiotic treatment may have underestimated the true prevalence of infection.

Trachoma is still the leading cause of preventable blindness worldwide, but the World Health Organisation (WHO) hopes to achieve global elimination of trachoma by the year 2020. A major part of their strategy is mass antibiotic administration aimed at reducing the prevalence of disease and the reservoir of infection with *C trachomatis*.¹ The ability to accurately measure the prevalence of *C trachomatis* infection in a village following mass antibiotic administration is critical for monitoring success of a treatment program and determining if further treatment is necessary.

DNA amplification tests are known to be highly sensitive and specific for the diagnosis of ocular chlamydial infection, and are superior to other laboratory methods such as tissue culture, antigen detection, and hybridisation.^{2,3} More recently, newer

nucleic acid amplification tests (NAATs) based on amplification of ribosomal RNA (rRNA) have been developed. Amplification of rRNA targets provides a potential advantage since bacterial rRNA is present at up to 10 000 times the copy number of genomic DNA and 1000 times that of plasmid DNA.⁴ This difference may become especially important when organisms are present in small numbers, such as in asymptomatic patients.⁵ Alternatively, Burton, *et al* have reported that a homebrew rRNA amplification test is far less sensitive than DNA amplification. They have suggested that this discrepancy may be due to the presence of dead or subviable *C trachomatis* that the rRNA test might not detect.⁶

Here, we compare the 16s rRNA-based APTIMA *C trachomatis* (ACT) assay (Gen-Probe, Inc., San Diego, CA, USA) to the DNA-based AmpliCor polymerase chain reaction (PCR) assay (Roche Molecular Systems, Branchburg, NJ, USA) for diagnosis of *C trachomatis* in ocular swabs taken from subjects living in a trachoma-endemic area. Both tests are commonly used for diagnosis of genital infection with *C trachomatis*.

MATERIALS AND METHODS

The sample population consisted of 56 consecutive subjects aged 0–10 years living in two hyper-endemic villages in the regional state of Amhara in Ethiopia. These villages had no history of prior antibiotic distributions. Children were selected because this age group is the most likely to harbor infection.⁷ Trained ophthalmic assistants supervised by experienced ophthalmologists performed the clinical examination. The upper tarsal conjunctiva of the right eye was examined using a 2.5× binocular loupe, and signs of trachoma were graded according to the WHO's simplified trachoma grading scheme for clinical activity: Normal, no signs of trachoma; TF, follicular trachomatous inflammation; TI, intense trachomatous inflammation.⁸ This grading scheme was selected in order to obtain a dichotomous definition of clinical activity for our analysis. The generally accepted threshold of five or more follicles of >0.5 mm was used as the threshold for defining active trachoma.⁹

For each subject, specimens were collected from the right upper tarsal conjunctiva for testing by ACT and AmpliCor. A swab was passed firmly across the conjunctiva along its full length three times, rotating approximately 120° between each pass. Specimens for the ACT assay were collected using swabs and transport tubes provided in the APTIMA Unisex Swab Specimen Collection Kit (Gen-Probe, Inc.). Specimens for the AmpliCor assay were collected using sterile Dacron-tipped

Abbreviations: ACT, APTIMA *C trachomatis*; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction; TF, follicular trachomatous inflammation; TI, intense trachomatous inflammation; WHO, World Health Organisation

plastic swabs (Fisher, Middletown, VA, USA) that were broken off into sterile 2.0 ml microcentrifuge tubes, and stored according to the Amplicor protocol. Each subject was assigned a random 5-digit identification number. The ACT specimen was collected first for even numbered subjects, and the Amplicor specimen for odd numbered subjects. Two types of field controls were obtained from a randomly selected 15% of children within the study group to evaluate the possibility of field contamination: a duplicate control (an Amplicor swab identical to and taken immediately following the initial Amplicor swab) and a negative field control (an Amplicor swab passed 2.5 cm above but not touching the conjunctiva). All specimens were stored at 4°C and transferred to -20°C within 24 hours of collection. Samples were transported to the University of California, San Francisco at 4°C and stored at -80°C before processing. Amplification and detection using ACT and Amplicor were performed according to the manufacturers' instructions. All specimens positive by ACT were submitted to confirmatory testing using the APTIMA Combo 2 (AC2) assay (Gen-Probe, Inc.) specific for 23s rRNA. Positive laboratory controls were included according to protocol for all assays.

To determine whether one test was more likely to be positive than another, a McNemar's test was performed (STATA 8.0, StataCorp LP, College Station, Texas, USA). A logistic regression model was used to evaluate whether the order in which swabs were taken affected the likelihood of a swab being positive.

RESULTS

The sample population consisted of 25 (45%) boys and 31 (55%) girls. Of 56 subjects total, 44 (78%) had clinically-active trachoma (TF and/or TI). 18/56 (32%) subjects were found to have TF, and 26/56 (46%) had TI. ACT detected *C trachomatis* in a significantly higher proportion of subjects compared to Amplicor. Of 56 subjects, ACT detected chlamydial infection in 35 (63%) subjects, and Amplicor 22 (39%) (McNemar's test, $p = 0.0002$). All 22 samples positive by Amplicor were also positive by ACT. Thirteen (23%) samples were ACT-positive but negative by Amplicor (table 1).

The results of ACT and Amplicor compared to clinical exam are shown in table 2. For each level of clinical activity (no active disease, TF, and TI), ACT yielded higher detection rates for *C trachomatis* infection than did Amplicor.

All 35 samples that were positive by ACT were re-tested using the AC2 assay as a confirmatory test. Of these, all 22 samples that were both ACT-positive and Amplicor-positive were also positive by AC2. Of the 13 discrepant results (ACT-positive and Amplicor-negative), 2 were positive by AC2 and 11 were negative. 10/13 (77%) of the discrepant samples were from individuals with TF or TI. The remaining three were from subjects with a normal exam. The two samples that were positive by both ACT and AC2 were from individuals with TI.

All duplicate control swabs were concordant with initial swab results and all negative field control swabs were negative. Test results were not affected by the order in which swabs were

taken. The effect on positivity of a swab being taken first was not statistically significant (odds ratio (OR) = 0.74; 95% confidence interval (CI), 0.15–3.81; $p = 0.72$).

The high prevalence of chlamydial infection observed in this study was consistent with previous studies in rural Ethiopia,^{10–12} with infection levels peaking among children aged 3–5 years and declining thereafter.

DISCUSSION

In this study, ACT detected *C trachomatis* infection significantly more often than Amplicor. ACT detected infection in all subjects that were positive by Amplicor, and also detected infection in 13 (23%) additional subjects. That 10 of these 13 discrepant results were from subjects with clinically active disease suggests that these individuals may have had chlamydial infection at levels below the detection ability of Amplicor. Our findings are consistent with prior, much larger studies of Gen-Probe's rRNA-based tests for detecting genital *C trachomatis*, which showed sensitivities of 100% and specificities of 98.3% to 100% using swab specimens,^{4, 5, 13–15} as well as increased sensitivity when compared to Amplicor.¹³

Alternatively, it is possible that the discrepant results are simply false positives of the ACT assay, and that ACT is in fact less specific. Only 2/13 (15%) of the ACT-positive, Amplicor-negative samples were confirmed as positive using the AC2 assay. Although this confirmation level is quite low, it is in accordance with recent large studies which showed ACT detected genital chlamydial infection more often than did AC2.^{16–18} AC2 in turn has been shown to be more sensitive than Amplicor.¹⁹ Our findings suggest that the same is true for the detection of ocular chlamydial infection. The 16s rRNA-based ACT assay appears to be more sensitive than the 23s rRNA-based AC2 assay, both of which are more sensitive than DNA-based Amplicor.

The results of our study are in contrast to those of a recent study in the Gambia by Burton, *et al* which found that their 16s rRNA-based test detected infection in fewer subjects than a DNA-based PCR assay.⁶ These authors suggested that an rRNA-based test may be more specific for established infections and could potentially discriminate metabolically-active organisms from dead or subviable organisms. The discrepancy between our findings and those of Burton *et al* may be due to the different 16s rRNA assays used (commercially available ACT assay developed by Gen-Probe versus homebrew assay). Detection could have also been affected by unaccounted differences between *C trachomatis* in Ethiopia versus the Gambia. For instance, variations in infectious load or molecular biology could conceivably cause discrepancies in rRNA-based test results.

The high proportion of ACT-positive, Amplicor-negative results (23%) raises the question of whether rRNA could be present in the absence of DNA. Although this is possible, it seems more likely that rRNA is simply more abundant than DNA, as has been suggested elsewhere.⁴ rRNA-based tests could also possess lower absolute thresholds of detection than PCR. Although current quantitative assays are unable to adequately assess this because they are less sensitive than PCR,^{6, 20, 21} perhaps in the future improved tests could help to clarify this issue. Regardless, it appears that rRNA-based NAATs have improved sensitivity over PCR. Chernesky, *et al* recently demonstrated with serial dilution assays that an rRNA-based test possessed 10-fold greater sensitivity than Amplicor.¹⁹

With the advent of newer NAATs for the detection of *C trachomatis*, we may be able to detect infections that were previously missed by PCR. A more sensitive indicator of infection may hold epidemiological significance by providing a more accurate assessment of the feasibility of eliminating

Table 1 Comparison of ACT and Amplicor for detection of *Chlamydia trachomatis*

rRNA Amplification test result	DNA Amplification test result		Total
	Amplicor ⁺	Amplicor ⁻	
ACT ⁺	22	13*	35
ACT ⁻	0	21	21
Total	22	34	56

*2 positive and 11 negative by confirmatory testing with AC2

Table 2 Comparison of ACT and Amplicor for detection of *Chlamydia trachomatis* by clinical group

	No. of subjects in clinical group	No. (%) positive in each clinical group by	
Clinical group		ACT	Amplicor
No active disease	12	6 (50)	3 (25)
Any clinical activity (TF and/or TI)	44	29 (66)	19 (43)
TF	18	9 (50)	5 (28)
TI ($\pm$ TF)	26	20 (77)	14 (54)
Total	56	35 (63)	22 (39)

ocular *C. trachomatis* infection with mass antibiotics. We are performing larger studies to further explore our findings and to correlate NAAT results with an expanded clinical grading system. Although the "gold standard" test for detection of ocular chlamydial infection remains to be determined, it is interesting to note that rRNA-based tests appear to offer significantly higher sensitivity. If rRNA-based tests are indeed more sensitive, then current DNA-based NAATs may not be detecting all true cases of ocular *C. trachomatis* infection. Past studies using PCR to assess prevalence of infectious trachoma following antibiotic treatment may have underestimated the true prevalence of infection and overestimated the effectiveness of antibiotic treatment. If this is the case, then elimination of trachoma may be more difficult than previously thought.

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Competing interests: JS has received funding from Roche and Gen-Probe for clinical trials and attendance of conferences, as well as donated test kits from both companies for research. JS has also received an unrestricted gift from Gen-Probe for chlamydial research.

Ethical approval: Ethical approval for this study was obtained from the Committee for Human Research of the University of California, San Francisco, and the Ethiopian Science and Technology Commission; the study was carried out in accordance with the Declaration of Helsinki.

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