Published in final edited form as:
Sex Transm Dis. 2013 May ; 40(5): 352–353. doi:10.1097/OLQ.0b013e3182841786.

Is There Evidence of the New Variant *Chlamydia trachomatis* in the United States?

Helen Won, BS[*,†], **Padmini Ramachandran, MS**[†], **Richard Steece, PhD**[‡], **Barbara Van Der Pol, PhD**[§], **Jeanne Moncada, MT**[¶], **Julius Schachter, PhD**[¶], and **Charlotte Gaydos, MS, DrPH**[*,‡]

[*]Division of Infectious Diseases, Department of Medicine, Johns Hopkins University, Baltimore, MD

[†]Department of Emergency Medicine, Johns Hopkins University, Baltimore, MD

[‡]Infertility Prevention Program, Centers for Disease Control and Prevention, Atlanta, GA

[§]Department of Epidemiology and Biostatistics, Indiana University, Bloomington, IN

[¶]University of California, San Francisco, CA

Abstract

A specific real-time polymerase chain reaction followed by melt curve analysis was developed for the detection of the Swedish variant (nv*CT*) strain of *Chlamydia trachomatis* (*CT*). Surveillance was performed on 476 *CT*-positive clinical specimens obtained from 15 laboratories around the United States using nucleic acid amplification test assays, which would not miss the nv*CT*. All were negative for nv*CT*; thus, there is no evidence of the nv*CT* in the United States.

Chlamydia trachomatis (*CT*) is the most common and most frequently reported sexually transmitted bacterial infection. Most infections are asymptomatic and often remain undiagnosed.[1] A new variant (nvCT) strain of *CT* has been previously been described in Sweden and Norway.[2] It has not been observed in Australia.[3] Most of the other detections of the nv*CT* outside Sweden and Norway have been shown to have epidemiologic linkages to Sweden.[4–9] No evidence of spread elsewhere has been reported.[10–13] The nv*CT* infections in different counties in Sweden have been reported to represent from around 10% of all *CT* infections up to 64% in Dalarna county in 2006 to 2007. In Södra Älvsborg County, among the 789 screening samples, 69 (8.8% positivity rate) were positive for wild-type chlamydia as detected by Roche CTM48. An additional 24 were identified using a laboratory-developed polymerase chain reaction (PCR) that could detect nv*CT*. Thus, 25% of a total of 93 *CT*-positive samples were nv*CT*.[7] As of yet, there has not been evidence of the nv*CT* in the United States, but surveillance has not been performed on a wide scale.

The nv*CT* is characterized by a 377-base-pair (bp) deletion in the cryptic plasmid, a common target for nucleic acid amplification test (NAAT) assays.[4,5] The Aptima Combo 2 test, Aptima *CT* (Genprobe, Inc., San Diego, CA), ProbeTec Strand Displacement Assay (Becton Dickinson, Sparks, MD), and RealArt *CT* Kit (Qiagen, West Sussex, UK) target either a different gene or a different region of the cryptic plasmid. These assays remain

Correspondence: Charlotte Gaydos, MS, DrPH, Division of Infectious Diseases, Department of Medicine, Johns Hopkins University, 855 N Wolfe St, Rangos Bldg, Rm 530, Baltimore, MD 21205. cgaydos@jhmi.edu.

These data were presented, in part, at the Proceedings of the Sixth Meeting of the European Society for Chlamydia Research. P-63, pp. 260, Aarhus, Denmark, July 1–4, 2008.

unaffected by the deletion but only identify the nv*CT* as *C. trachomatis* and do not distinguish it as the nv*CT*.[2,4,5] However, other older NAAT assays (Roche Cobas Amplicor, Roche TaqMan48, and the earlier version Abbott m2000 assay) that target the portion of the plasmid with the deletion produced false-negative results and were unable to detect the nv*CT*.[4,5]

Our objective was to develop a specific real-time PCR for the nv*CT*, followed by confirmatory high-resolution melt analysis (HRMA) for the detection of the nv*CT* and perform surveillance in the United States. As a pilot surveillance study, *C. trachomatis* clinical samples tested by existing NAAT assays that would not miss detection of the nv*CT* strain as a *CT* strain, from across the United States, were obtained and tested using our PCR assay. Such assays would not differentiate *CT*, however, as a nv*CT* but indicate it as positive for *CT*. Samples that had been collected for 3 different years, from 2008 to 2010, were obtained for this study as de-identified remnant samples and were from the following states: Ohio, North Carolina, Massachusetts, Indiana, Washington, Kansas, Georgia, Montana, Delaware, California, Maryland, Tennessee, Michigan, New Jersey, and Virginia. These states were volunteers wishing to participate after a request went out to the State Health Laboratories participating in the Infertility Prevention Program of the Centers for Disease Control and Prevention.

A total of 476 swab and urine samples previously tested positive by Aptima Combo 2 (Gen-Probe, Inc., San Diego, CA) or Becton Dickinson (Sparks, MD) for *C. trachomatis* were tested using our assay. The nv*CT* DNA extract for use as a positive control was kindly provided by Dr Kenneth Persson from Malmo, Sweden. Samples were de-identified for research purposes. The study was approved by The Johns Hopkins University institutional review board.

DNA was extracted from the samples using the Roche MagNA Pure robotic instrument (Roche Diagnostics, Indianapolis, IN). A specific primer pair flanking the deletion region, chlamydia mutant forward (CtMF) and chlamydia mutant reverse (CtMR) (Table 1), modified from a previously described primers,[3,4] and a novel Taqman 26-bp probe, which covered the sequences on either side of the deletion (Table 1) were designed and used. The assay was designed to only generate a positive signal when the probe binds to the nv*CT* target. Polymerase chain reaction included the following: 50 μL total volume 10 μL of DNA template, 1 μL of 50 μM CtMF and CtMR primers, 12.8 μL molecular-grade water, 0.2 μL of 50 μM probe, and 25 μL of 2× Taqman Universal PCR mix (PE Applied Biosystems, Foster City, CA) The cycling conditions were 2 minutes preincubation at 50°C, denaturation at 95°C for 10 minutes and 50 repeats at 95°C for 15 seconds, and annealing/extension at 60°C for 60 seconds.

Subsequently, another specific PCR was performed, without use of the probe, but coupled with melt curve analysis of the amplicon. Melt analysis was performed on the Lightscanner instrument (Idaho Technology, Salt Lake City, UT) for confirmation of a possible nv*CT*. A 10-μL volume of PCR mixture comprised 2 μL of DNA template, 1 μL of 1.5 μM CtMF and CtMR primer, 2 μL molecular-grade water, and 4 μL of 2× Universal PCR mix and LC Green dye (Idaho Technology) was tested in triplicate in a Geneamp Thermocycler (PE Applied Biosystems) using denaturation at 95°C for 30 seconds, followed by 45 cycles at 95°C for 30 seconds and annealing/extension at 60°C/72°C for 60 seconds, and 1 cycle at 95°C for 30 seconds and 28°C for 30 seconds. Each post-PCR sample amplicon was subjected to HRMA on the LightScanner instrument, version 2.0. Melting temperatures ranged from 60°C to 95°C. Fluorescence data acquisition was obtained for every 0.1°C increase in temperature. Confirmatory melting profiles generated by HRMA for the nv*CT* and the wild-type *CT* were 73.19°C and 82.79°C, respectively.

Of the 476 samples tested, all 476 were negative for the nv*CT* using the real-time PCR with probe detection and PCR coupled with HRMA. The Chlamydia-positive samples were mostly from laboratories serving sexually transmitted disease and family planning clinics. Continued surveillance for this variant may not be as necessary in the future because the older assays are being phased out of use. The use of the real-time PCR coupled with melt analysis confirmation not only allowed detection of the nv*CT* but may also allow for detection of an unknown or emerging variant of *C trachomatis*, if different melt curve temperature results are detected.

Although many of the currently used NAATs, such as m2000 PCR (Abbott Molecular) and COBAS TaqMan *CT* v2.0, will not miss the nv*CT*,[14,15] there are reasons for some continued surveillance because of the possibility of new variants that may arise. US public health laboratories perform approximately 3.6 million *CT* tests per year.[16] Most (64.4%) are NAATs, many of which target the *CT* plasmid, and NAAT assays are increasingly being used.[16] Monitoring of test capacity for detection of possible new molecular variants of *CT* should continue.

Thus far, surveillance has demonstrated that the nv*CT* persists in Sweden[7,8] and Norway.[9] There has been no evidence of spread to Australia,[3] England,[10,11] Wales,[11] Ireland,[12] or the Netherlands[13]; and we, too, find no evidence that the new variant has spread to the United States.

Acknowledgments

We thank all the laboratories that provided specimens for testing. Funded, in part, by U54EB007958 and HPTN NIAID/NIH U01-AI 068613.

References

1. CDC. Sexually Transmitted Disease Surveillance, 2010. Atlanta, GA: Department of Health and Human Services; 2011.
2. Marions L, Rotzen-Ostlund M, Grillner L, et al. High occurrence of a new variant of *Chlamydia trachomatis* escaping diagnostic tests among STI clinic patients in Stockholm, Sweden. Sex Transmit Dis. 2008; 35:61–64.
3. Stevens MP, Tan SE, Horvath L, et al. Absence of a *Chlamydia trachomatis* variant, harboring a deletion in the cryptic plasmid, among two Melbourne cohorts: Clients of a STI clinic and antenatal screening patients. Commun Dis Intell Q Rep. 2008; 32:77–81. [PubMed: 18522308]
4. Ripa T, Nilsson PA. A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: Implications for use of PCR diagnostic tests. Euro Surveill. 2006; 11:E061109.2. [PubMed: 17213548]
5. Ripa T, Nilsson PA. A *Chlamydia trachomatis* strain with a 377-bp deletion in the cryptic plasmid causing false-negative nucleic acid amplification tests. Sex Transm Dis. 2007; 34:255–256. [PubMed: 17483723]
6. Hermann B. A new genetic variant of *Chlamydia trachomatis*. Sex Transmit Infect. 2007; 83:253–254.
7. Söderblom T, Blaxhult A, Fredlund H, et al. Impact of a genetic variant of *Chlamydia trachomatis* on the national detection rates in Sweden. Euro Surveill. 2006; 11:E061207.1. [PubMed: 17213562]
8. Unemo M, Oleen P, Agne-Stadling I, et al. Experiences with the new genetic variant of *Chlamydia trachomatis* on Orebro county, Sweden—Proportion, characteristics, and effective diagnostic solution in an emergent situation. Euro Surveill. 2007; 12:E5–E6. http://www.eurosurveillance.org/em/v12n04/1204-223.asp. [PubMed: 17991387]
9. Moghaddam A, Reinton N. Identification of the Swedish *Chlamydia trachomatis* variant among patients attending a STI clinic in Oslo, Norway. Euro Surveill. 2007; 12:E070208 3.

10. Sillis M, Skidmore S, Mallinson H, et al. No evidence of the *Chlamydia trachomatis* variant in the UK. Sex Transm Infect. 2007; 83:488–489. [PubMed: 17715158]

11. Alexander S, Ison C. Is new variant *Chlamydia trachomatis* present in England and Wales? Sex Transmit Dis. 2008; 84:29–31.

12. Lynagh Y, Crowley B, Walsh A. Investigation to determine if newly-discovered variant of *Chlamydia trachomatis* is present in Ireland. Euro Surveill. 2007; 12:E070201 2. [PubMed: 17370946]

13. De Vries HJ, Catsburg A, van der Helm JJ, et al. No indication of Swedish *Chlamydia trachomatis* variant among STI clinic visitors in Amsterdam. Euro Surveill. 2007; 12:E070208 3. [PubMed: 17370951]

14. Moller JK, Pedersen LN, Persson K. Comparison of the Abbott RealTime CT New Formulation Assay with two other commercial assays for detection of wild-type and new variant strains *of Chlamydia trachomatis*. J Clin Microbiol. 2010; 48:440–443. [PubMed: 20007394]

15. Hasad R, Fredlund H, Unemo M. Evaluation of the new COBAS TaqMan CT test v2.0 and impact of the proportion of the new variant *Chlamydia trachomatis* by the introduction of diagnostics detecting new variant *C. trachomatis* in Orebro county. Sweden Sex Transm Dis. 2007; 85:190–193.

16. Dicker LW, Mosure DJ, Steece R, et al. Testing for sexually transmitted diseases in U.S. Public health laboratories in 2004. Sex Transm Dis. 2007; 34:41–46. [PubMed: 16735955]

TABLE 1

Primer and Probe Sequences for Real-Time nv*CT* PCR

CtMF primer	5′-TCCGGATAGTAGATTATAGAGACT-3′
CtMR primer	5′-CCTAAAAGTGTTTTTTCTGGC-3′
nv*CT* probe	6FAM-AAG GGA TCC GT*T TGT TCT GGG GAA GA-MGBNFQ

*A deletion location of 377 bp.