HHS Public Access

Author manuscript

Sex Transm Dis. Author manuscript; available in PMC 2023 June 01.

Published in final edited form as:

Sex Transm Dis. 2022 June 01; 49(6): 448–452. doi:10.1097/OLQ.0000000000001617.

Chlamydia trachomatis variants escaping detection in the Aptima Combo 2® assay in the United States

Samantha S. Katz, PhD[1,*], **Damien C. Danavall, MS**[1], **Monica R. Morris, MPH**[1], **Bridgett P. Herrod, MPH**[1], **Suzanne E. Dale, PhD**[2], **Melinda B. Nye, PhD**[2], **Ellen N. Kersh, PhD**[1], **Robert D. Kirkcaldy, MD**[1], **Brian H. Raphael, PhD**[1]

[1]Division of STD Prevention, Centers for Disease Control and Prevention, Atlanta, GA

[2]Center for Esoteric Testing, Laboratory Corporation of America, Holdings, Burlington, NC

Abstract

Background: The Aptima Combo 2® (AC2) assay manufactured by Hologic, Inc. detects *Neisseria gonorrhoeae* (NG) and/or *Chlamydia trachomatis* (CT) in urogenital and extragenital specimens by targeting either a 16S rRNA (NG) or 23S rRNA (CT) region. In 2019, a mutation (C1515T) in the 23S rRNA region was reported to cause false negative/equivocal results in specimens collected in Finland. Specimens containing this variant (Fl-nvCT) were also discovered internationally. Working with specimens submitted to a large commercial laboratory, we sought to determine if this variant was also present in the United States.

Methods: A subset (N=401) of specimens tested with the AC2 assay collected during a five-week period in late 2019/early 2020 were evaluated using an updated AC2 assay.

Results: While the FI-nvCT variant was not detected within this specimen panel, two CT variants containing 23S rRNA mutations (A1518G, G1526A) were identified. The updated AC2 assay targeting an additional region of the 23S rRNA detected both of these variants. A retrospective study of >18 million AC2 results tested between 2018–2019 did not display a decrease in CT positivity.

Conclusions: Although we did not detect the Fl-nvCT variant among US specimens, we show evidence that the low occurrence of similar diagnostic escape mutants can be detected with an updated AC2 assay using multiple 23S rRNA targets.

Short Summary

Chlamydia trachomatis diagnostic escape variants were detected in the United States using a redesigned Aptima Combo 2 assay, which also correctly identified specimens previously identified as false-negative or equivocal.

[*]Correspondence and reprints: Samantha S. Katz, PhD, Centers for Disease Control and Prevention, 1600 Clifton Rd NE Mailstop H23-3, Atlanta, GA 30329; SSKatz@cdc.gov; phone: 404.639.3710; fax: 404.639.4664.

Disclosure: None of the authors have conflicts of interest to disclose.

Keywords

Chlamydia trachomatis ; 23S rRNA; Aptima Combo 2; false negative; diagnostic escape mutant

Introduction

Chlamydia trachomatis (CT) infection is a nationally notifiable infectious disease in the United States (US), with 1,808,703 cases reported in 2019 (1). Chlamydial infection can cause significant reproductive sequelae in women including pelvic inflammatory disease (PID), ectopic pregnancy, and tubal factor infertility (TFI) (2, 3). The Centers for Disease Control and Prevention (CDC) recommends the use of nucleic acid amplification tests (NAAT) for molecular detection of CT (4). The Aptima Combo 2® assay (AC2), manufactured by Hologic, Inc., is a commercially available NAAT that is widely used to co-detect CT and *Neisseria gonorrhoeae* (NG), targeting 23S ribosomal RNA (rRNA) and 16S rRNA in these bacteria, respectively. Detection of CT and/or NG is distinguished based on the kinetic profile of light emitted by chemiluminescent labels of specific probes in a transcription-mediated amplification (TMA) reaction. For specimens with only a CT signal, results are determined, in part, by relative light units (RLU) with the following threshold values: positive (RLU ≥100), negative (RLU <25), or equivocal (RLU ≥25–100).

In 2019, researchers in Finland reported the identification of CT false-negative/equivocal clinical specimens using the AC2 assay (5). Sequencing analysis of the CT 23S rRNA gene confirmed the failure to detect CT in these specimens occurred due to a C1515T mutation located in the assay's probe-binding region (5, 6). This diagnostic-escape variant, designated FI-nvCT, was also detected in Sweden (7), Denmark (8), and Norway (9). Subsequent studies have also revealed the presence of other diagnostic escape mutants (C1514T, C1522T, and G1523A) (8–10). Notably, a study in England conducted during 2019 failed to detect the Fl-nvCT variant but the C1514T and G1523A variants were detected (10, 11).

Considering the potential for a prevalence of the FI-nvCT variants circulating in the US, we collected potentially false-negative or equivocal specimens initially tested with the AC2 assay submitted to a large commercial testing laboratory. Additional molecular characterization of these specimens was conducted at CDC. In response to the discovery of the Fl-nvCT and other variants, Hologic developed an updated AC2 assay which included an additional target allowing detection of additional mutations present in the 23S rRNA target region (12). When evaluated with more than 1000 clinical specimens and 225 analytical samples, this updated assay demonstrated high clinical sensitivity and specificity for the co-detection of CT wild-type or variant 23S rRNA with NG (12). In this study, we sought to determine the prevalence of the Fl-nvCT and/or other variants in the US and to determine the suitability of the updated AC2 assay, which we obtained as a research-use only (RUO) kit, for detection of CT 23S rRNA variants.

Materials and Methods

Retrospective positivity rate analysis

Retrospective AC2 test result data from Laboratory Corporation of America Holdings (Labcorp) was examined to assess trends in the CT positivity rate over time. The weekly positivity rate for 18,476,856 specimens tested by AC2 during December 24, 2017-December 25, 2019 was determined and plotted by annual quarters using R (13).

Selection of specimens tested using the AC2 assay

Labcorp identified a subset of specimens tested using the AC2 at its Burlington, North Carolina (NC) location over a 5-week period during late December 2019 to early February 2020 for further molecular characterization at CDC. Approximately 470,915 specimens were tested by AC2 using the Panther® (Hologic, Inc.) instrument during this timeframe. Only NG-negative specimens were selected. Based on previous studies examining the prevalence of the Fl-nvCT variant in other countries, CT-negative or equivocal specimens with RLU ≥15–99 were considered to most likely include potential variants (5, 7, 9). All specimens (N=50) within this group were selected for further characterization (see Supplemental Figure 1 for testing workflow). A further cohort of 300 CT-negative specimens with RLU <15 were additionally selected, as were 51 specimens (CT-positive N=47, with N=2 each CT-negative or CT-equivocal) with RLU ≥100. Specimen types included urine, rectal, endocervical, vaginal, and oropharyngeal swabs. De-identified patient information, including geographic (state) location of collection, anatomical site, patient gender and age, and date of collection was also provided. Specimens included in this study represented individuals in nearly 30 states; approximately half (~51%) were from Virginia, Illinois, Georgia, South Carolina, and North Carolina. This study was determined to not be human subject research through review by the National Center for HIV/AIDS, Viral Hepatitis, STD, and Tuberculosis Prevention.

Molecular characterization of selected specimens

Genomic DNA (gDNA) was isolated from all specimens (N=401) using 200 μL aliquots processed with the QIAsymphony DSP DNA Mini Kit (Qiagen, Inc., Germantown, MD) on the QIAsymphony (Qiagen, Inc.) automated platform. All specimens were tested using a lab-developed duplex real-time PCR (CT PCR) targeting the CT cryptic plasmid and human ribonuclease P (RNP) as an internal control for a valid clinical specimen on a Rotor-Gene real-time PCR instrument (Qiagen, Inc.) as described previously (14). This in-house assay was previously validated on rectal swab specimens with a limit of detection of ≤100 copies per reaction and analytic sensitivity and specificity of the CT target of 93% and 100%, respectively (14).

All specimens from the ≥15–99 RLU category (N=50) and a subset (N=5) from each of the other RLU categories were tested using a nested end-point PCR assay targeting a portion of the CT 23S rRNA gene with primers previously published (6). Sequencing of the 23S variable region 2 (V2) in purified amplicons proceeded using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Products were analyzed on an Applied Biosystems 3500xL Genetic Analyzer. Reads were assembled

and analyzed in Geneious Prime software (Biomatters Ltd., Auckland, New Zealand) for sequence determination.

Additionally, a total of 64 specimens consisting of the entire ≥15–99 RLU category, five from the <15 RLU category, and nine from the ≥100 RLU category (including the five sequenced as described above along with the four AC2 CT negative or equivocal specimens), were re-tested on the Hologic Panther® platform using the updated AC2 assay, which we obtained as an RUO formulation as the updated formulation was not yet FDA-cleared. Results between the original *in vitro* diagnostic (IVD) and the updated RUO AC2 assays were compared to assess the performance of this new assay on these specimens.

Results

No change in CT positivity rate of specimens tested in a large commercial US laboratory

Among specimens tested during December 24, 2017–December 25, 2019, the average weekly CT positivity rate was 5.5% with a range of 4.6–6.6% (Fig 1). We did not observe any downward trends in positivity as may be expected if large numbers of false-negative tests were occurring due to widespread circulation of diagnostic escape mutants. Notably, weeks with high CT positivity rates (>6.5%) were observed and may likely be associated with repeat testing of specific specimens (e.g., retesting of specimens with an initial equivocal result).

Detection of false negative/equivocal specimens

A total of 401 remnant specimens were collected for further evaluation. These specimens were grouped into three different categories based on their initial RLU signal in the AC2 assay: RLU <15, RLU ≥15–99, and RLU ≥100. For each category, the majority of specimens were collected from females (Table 1). The largest proportion of specimens in the <15 RLU category were vaginal swabs while both the ≥15–99 and ≥100 RLU categories had large proportions (~40%) of specimens from unknown anatomical sites. The median ages of individuals from whom specimens were collected was ≤30.

DNA was isolated from all 401 remnant specimens and tested with a lab-developed real-time PCR to determine the suitability of this genetic material for downstream applications, including Sanger sequencing. As expected, all CT-negative specimens with low RLU values (i.e., <15 RLU category) were CT PCR negative (a single specimen was determined to be invalid due to a failure to detect human DNA) (Fig 2). Despite previous AC2 results from specimens in the ≥100 RLU category demonstrating CT positivity in 47/51 specimens (92%), we were only able to detect CT DNA by real-time PCR in 17 of those 47 (36.2%). In the ≥15–99 RLU category, which included the range of RLU values with the greatest probability of containing diagnostic-escape variants, we detected seven CT-positive specimens by real-time PCR, indicating those specimens might contain genetic variance within the 23S rRNA target region.

We attempted Sanger sequencing of 23S rRNA AC2 assay target region for all 50 specimens in the ≥15–99 RLU category group. As controls, we attempted sequencing in a subset of specimens from the <15 RLU (N=5) and ≥100 RLU (N=5) categories. Not surprisingly,

there was no amplification and no sequencing results for those tested from the <15 RLU category. All five specimens from the ≥100 RLU category revealed wild-type 23S rRNA sequences. Of the 50 specimens from the ≥15–99 RLU category, six specimens (which were also CT PCR positive) were successfully sequenced. Analysis of the genetic sequence in those six specimens revealed four with wild-type 23S rRNA sequences and two with variations (A1518G and G1526A) in the AC2 assay target region.

Evaluation of an updated AC2 assay for detection of CT variants

We further evaluated the updated RUO AC2 assay using the 60 remnant specimens initially tested with the commercially available IVD AC2 assay and on which sequencing was attempted in this study. In addition, four CT-negative/equivocal specimens from the ≥100 RLU category were examined due to their RLU values from the IVD AC2 assay (between 118 and 226) which often is associated with an algorithmic determination of positive CT status. Specimens in the <15 RLU category again tested negative for CT using the RUO reagents (Fig 3). One of the CT-equivocal specimens from the ≥100 RLU category was classified as CT-positive when re-tested with the updated AC2 reagents (initial RLU of 243 whereas the updated RLU using the updated reagents was 779) and all of the initially CT-positive specimens producing the same results in the RUO assay. The other CT-equivocal specimen from this category became CT-negative as determined by the assay's algorithm (original RLU of 130 and new RLU of 123) while the two CT-negative specimens from this category remained negative (original RLU value of 118 for both and new RLUs of 10 or 11). In the ≥15–99 RLU category, all 50 specimens were re-tested and, of these, nine originally CT-negative and 13 originally CT-equivocal were CT-positive upon re-test using the RUO assay. These 22 encompassed various specimen types including endocervical, vaginal, oropharyngeal, and rectal swabs as well as urine and included the six real-time PCR CT-positive specimens that were successfully sequenced. The seventh CT PCR positive specimen (specimen type not provided) from this category which we were unable to sequence remained CT-negative using the updated AC2. In total, 23 specimens CT-negative/equivocal by the IVD AC2 assay were CT-positive using these RUO reagents.

We further explored the specimens in the ≥15–99 RLU category that were re-tested using the RUO AC2 assay by stratifying across RLU values produced in the initial IVD AC2 assay. Among the specimens originally testing negative for CT, most (81.3%) of those with initial RLU values between 15–32 were also negative in the RUO assay (Table 2). The proportion of initially CT-negative specimens testing positive in the RUO assay did not increase with higher RLU values from the IVD assay. Notably, none of the specimens in the ≥15–99 RLU category originally testing equivocal for CT were found to be equivocal in the RUO assay; most (≥70%) of these specimens tested positive in the RUO assay.

Discussion

A retrospective analysis of AC2 test results from over 18 million specimens tested between approximately 2018–2019 failed to demonstrate a decrease in overall positivity rate. Of the 470,915 clinical specimens tested at Labcorp's Burlington, NC facility using the AC2 during a 5-week period from late December 2019 – early February 2020, a total of 401 specimens

were selected for further characterization. Analysis of 50 of these specimens, which were selected based on AC2 test results placing them in the ≥15–99 RLU category, yielded seven CT-positive specimens using CT PCR which were not detected as such using the IVD AC2 assay. Upon genetic sequencing of a portion of the 23S rRNA V2 region, we confirmed two diagnostic escape CT 23S variants (A1518G and G1526A) that have not been previously reported. While we did not detect any Fl-nvCT variants among US specimens, these variants add to other known AC2 diagnosis escape mutants described in other recent studies (8, 9). Similar to our findings, researchers in England did not detect the Fl-nvCT variant among specimens collected for testing during a five-month period in 2019 (10, 11). However, two other variants (C1514T, G1523A) were detected among false CT-negative/equivocal specimens.

Diagnostic escape mutants have been documented with other commercial NAAT assays to detect CT. A variant with a deletion in the CT cryptic plasmid was initially detected in 2006 among false-negative specimens in Sweden tested with versions of NAATs including the Cobas TaqMan CT test (Roche) and Abbott m2000 that were commercially available at the time (15, 16). The Swedish variant was not detected in a study examining CT-positive clinical specimens from the US that were initially tested with a separate NAAT that did not target the cryptic plasmid (17). The discovery of diagnostic escape mutants underscores the selective advantage of CT to evade detection and treatment. Although not all variants become widespread, their detection highlights the risk of false-negative results using assays that detect a single target.

An updated AC2 assay was developed that uses two targets with the goal of better detecting target region variants (12). This assay successfully detected both variants identified in this study (A1518G and G1526A). During the timeframe of this study the updated AC2 assay became FDA-cleared, though for the work presented here this kit was used under RUO labeling. In our evaluation, we demonstrated it also detected CT in specimens with an initial CT-negative/equivocal AC2 result including those with CT PCR positive results and other specimens where we were unable to conduct sequencing and/or detect CT via real-time PCR. While both AC2 assay formats (IVD and RUO) demonstrated higher CT positivity than using CT PCR and/or Sanger sequencing, this finding is not particularly surprising given the nature of our approach. Isolation of DNA from clinical specimens is problematic due to a number of parameters, and the lower yield of CT-positive samples by our methods highlights the challenges we faced with not only detection but also with determination of the genetic sequence. And whereas we conducted DNA isolation followed by real-time PCR targeting cryptic plasmid DNA (CT PCR) or the 23S rRNA gene (Sanger sequencing), the AC2 assay combines specimen processing for nucleic acid followed by target capture, transcription-mediated amplification (TMA), and dual kinetic assay (DKA) technology which likely results in higher sensitivity for CT.

There are some limitations to our findings. First, the specimens tested in this study were collected from individuals from states in the mid-Atlantic region of the US. This could yield disproportionate findings on the presence of CT variants, especially if such variants circulated in other US regions. Due to challenges associated with procuring specimens during the timeframe of this study, the anticipated numbers of specimens to be included for

a more robust analysis was drastically reduced. Specimens were selected over a five-week period which may have limited the ability to detect low frequency CT variants. Also, due to the nature of our approach and the challenges we faced with detection of CT DNA, it is possible we failed to detect other or additional CT variants.

The identification of the Fl-nvCT variant prompted additional studies internationally to assess the local prevalence of this and other variants. In addition, an updated AC2 assay was developed by the manufacturer which demonstrates improved suitability for detection of several CT variants, including the two identified in the current study as well as four others identified previously (12), and which otherwise demonstrates comparable performance to its predecessor. As this updated assay became FDA-cleared during the timeframe of this study, circulation of strains containing these 23S rRNA mutations should be detected using this new formulation. Nonetheless, laboratory quality practices, such as ongoing positive rate analysis, reflex/retesting of specimens with equivocal results using alternative tests, and other quality management practices, are key to detecting false test results with any diagnostic assay. Our work also highlights an important partnership between a public health entity and a commercial testing laboratory which allowed screening of a very large number of specimens for a low prevalence but potentially significant CT variant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

Thank you to the Labcorp Specimen Access Team members for sample procurement and data collection functions. We also thank Hologic, Inc. for access to the now FDA-cleared updated AC2® kits, used in this study as research-use only.

All sources of support:

This publication was funded by the Centers for Disease Control and Prevention (CDC).

References

1. Prevention CfDCa. Sexually Transmitted Disease Surveillance 2019. Atlanta: U.S. Department of Health and Human Services; 2021.
2. Oakeshott P, Kerry S, Aghaizu A, et al. Randomised controlled trial of screening for Chlamydia trachomatis to prevent pelvic inflammatory disease: the POPI (prevention of pelvic infection) trial. BMJ. 2010;340:c1642. [PubMed: 20378636]
3. Westrom L, Joesoef R, Reynolds G, Hagdu A, Thompson SE. Pelvic inflammatory disease and fertility. A cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic results. Sex Transm Dis. 1992;19(4):185–92. [PubMed: 1411832]
4. Centers for Disease C , Prevention. Recommendations for the laboratory-based detection of Chlamydia trachomatis and Neisseria gonorrhoeae−−2014. MMWR Recomm Rep. 2014;63(RR-02):1–19.
5. Rantakokko-Jalava K, Hokynar K, Hieta N, et al. Chlamydia trachomatis samples testing falsely negative in the Aptima Combo 2 test in Finland, 2019. Euro Surveill. 2019;24(22).
6. Hokynar K, Rantakokko-Jalava K, Hakanen A, et al. The Finnish New Variant of Chlamydia trachomatis with a Single Nucleotide Polymorphism in the 23S rRNA Target Escapes Detection by the Aptima Combo 2 Test. Microorganisms. 2019;7(8).

7. Unemo M, Hansen M, Hadad R, et al. Finnish new variant of Chlamydia trachomatis escaping detection in the Aptima Combo 2 assay also present in Orebro County, Sweden, May 2019. Euro Surveill. 2019;24(26).
8. Hadad R, Jensen JS, Westh H, et al. A Chlamydia trachomatis 23S rRNA G1523A variant escaping detection in the Aptima Combo 2 assay (Hologic) was widespread across Denmark in July-September 2019. APMIS. 2020;128(6):440–4. [PubMed: 32202687]
9. Johansen TB, Klovstad H, Rykkvin R, et al. The 'Finnish new variant of Chlamydia trachomatis' escaping detection in the Aptima Combo 2 assay is widespread across Norway, June to August 2019. Euro Surveill. 2019;24(42).
10. Roberts DJ, Davis GS, Cole MJ, et al. Prevalence of new variants of Chlamydia trachomatis escaping detection by the Aptima Combo 2 assay, England, June to August 2019. Euro Surveill. 2019;24(38).
11. Cole MJ, Davis GS, Fifer H, et al. No widespread dissemination of Chlamydia trachomatis diagnostic-escape variants and the impact of Neisseria gonorrhoeae positivity on the Aptima Combo 2 assay. Sex Transm Infect. 2021.
12. Unemo M, Hansen M, Hadad R, et al. Sensitivity, specificity, inclusivity and exclusivity of the updated Aptima Combo 2 assay, which provides detection coverage of the new diagnostic-escape Chlamydia trachomatis variants. BMC Infect Dis. 2020;20(1):419. [PubMed: 32546213]
13. Team RC 2019;Pages. Accessed at https://www.R-project.org/.
14. Woodson EN, Katz SS, Mosley SS, et al. Use of real-time PCR as an alternative to conventional genotyping methods for the laboratory detection of lymphogranuloma venereum (LGV). Diagn Microbiol Infect Dis. 2021;101(4):115532. [PubMed: 34571353]
15. Ripa T, Nilsson P. A variant of Chlamydia trachomatis with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. Euro Surveill. 2006;11(11):E061109 2.
16. Soderblom T, Blaxhult A, Fredlund H, Herrmann B. Impact of a genetic variant of Chlamydia trachomatis on national detection rates in Sweden. Euro Surveill. 2006;11(12):E061207 1.
17. Won H, Ramachandran P, Steece R, et al. Is there evidence of the new variant Chlamydia trachomatis in the United States? Sex Transm Dis. 2013;40(5):352–3. [PubMed: 23588122]

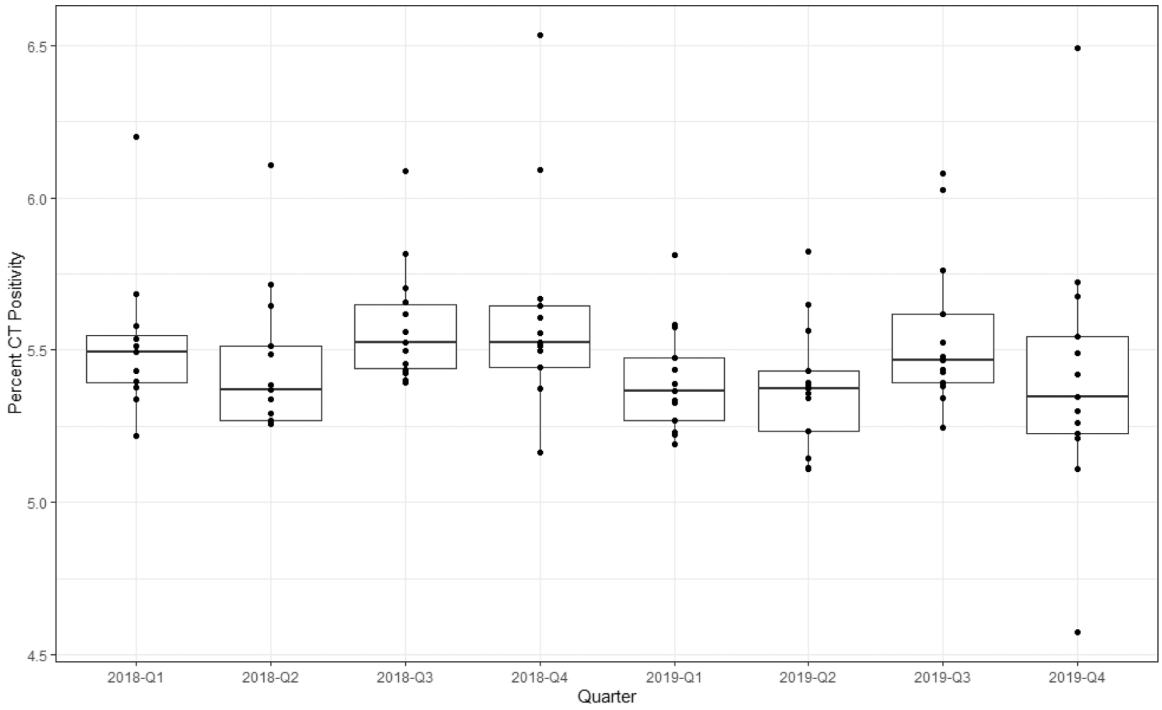

Figure 1. Retrospective data analysis of *Chlamydia trachomatis* (CT) positivity rate across a two-year period.

The positivity rate for CT across each three-month quarter is plotted, with each data point representing the average weekly positivity rate. Quarter 1 (Q1), Quarter 2 (Q2), Quarter 3 (Q3), Quarter 4 (Q4).

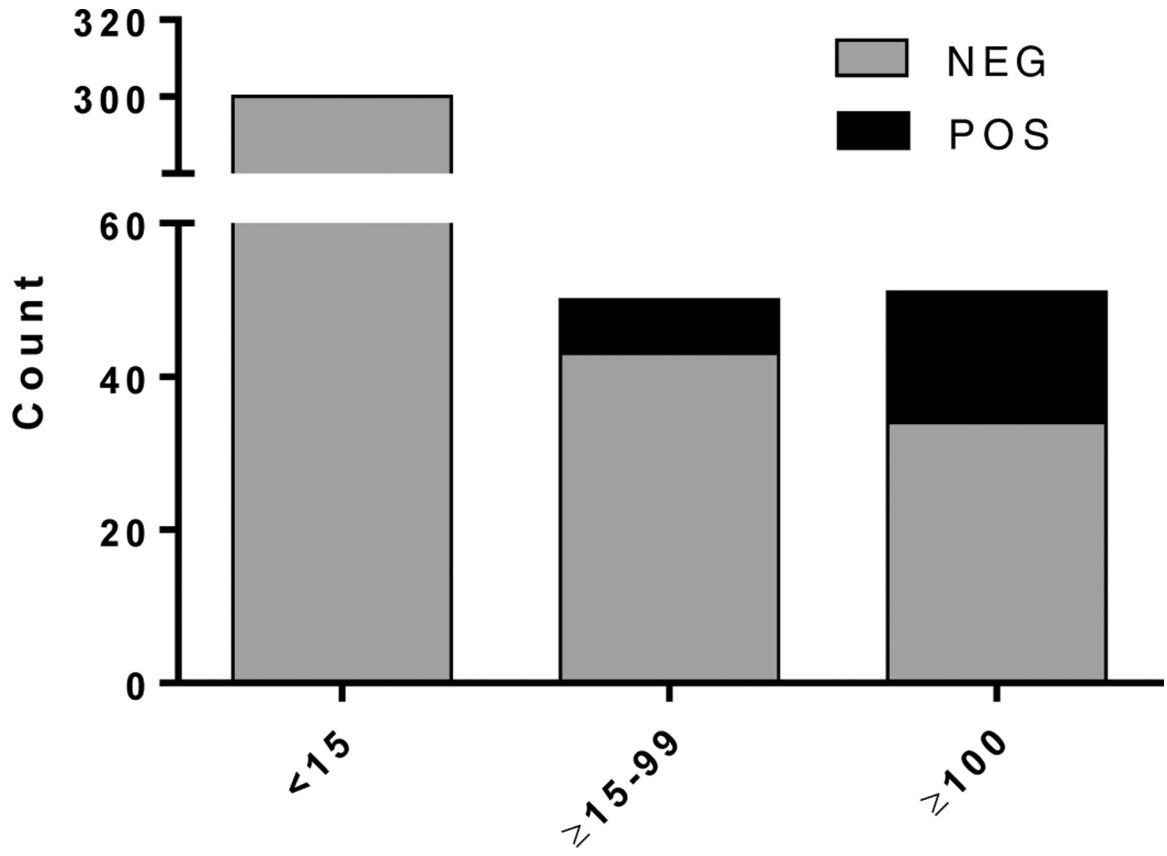

Figure 2. Real-time PCR results based on positive (POS) or negative (NEG) detection of cryptic plasmid DNA.

Results are stratified by relative light unit (RLU) category, including <15 RLU (N=299; an additional specimen was invalid due to negative detection of human DNA), ≥15–99 RLU (N=50), and ≥100 RLU (N=51). All specimens from the <15 RLU category were CT-negative by real-time PCR and the AC2 assay. Seven (14%) of the ≥15–99 RLU category specimens, which were CT-negative or equivocal by AC2, were positive for CT DNA by real-time. From the ≥100 RLU category CT DNA was detected in 17/47 (36.2%) of the AC2 CT-positive specimens and in none (0/4) of the CT-negative or equivocal specimens from that category.

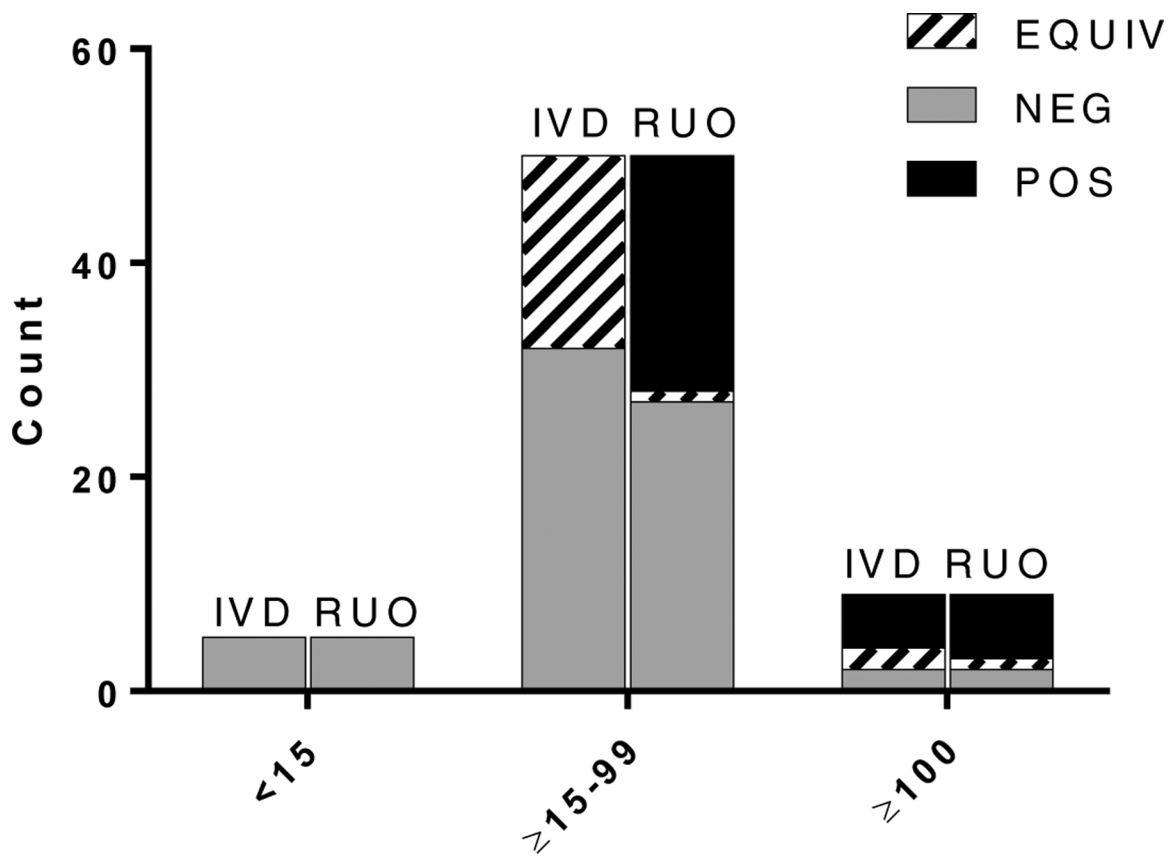

Figure 3. Aptima Combo 2® (AC2) assay results, stratified by RLU category.

Results from the research-use only (RUO) and commercially available *in vitro* diagnostic (IVD) assays are shown based on assay classification as CT-negative (NEG), positive (POS), or equivocal (EQUIV). <15 RLU (N=5), ≥15–99 RLU (N=50), and ≥100 RLU (N=9)

Table 1.

Patient characteristics of remnant specimens (N=401) used in this study collected over a five-week period (December 2019 - February 2020) for Aptima Combo 2® *Chlamydia trachomatis* testing in the United States.

RLU Category	No. of Specimens	Gender (N [% per RLU category])			Age Median	Specimen Type (N [% per RLU category])					
		M	F	Not provided		Vaginal	Endocervical	Pharyngeal	Rectal	Urine	Unknown
<15	300	47 *(15.7)*	252 *(84)*	1 *(0.3)*	30	126 *(42)*	17 *(6)*	36 *(12)*	5 *(2)*	39 *(13)*	74 *(25)*
15–99	50	15 *(30)*	35 *(70)*	0 *(0.0)*	24.5	7 *(14)*	3 *(6)*	2 *(4)*	5 *(10)*	12 *(24)*	21 *(42)*
>100	51	16 *(31.4)*	35 *(68.6)*	0 *(0.0)*	24	8 *(16)*	7 *(14)*	4 *(8)*	2 *(4)*	10 *(20)*	20 *(39)*
Total (% of total)	401	78 (*19.5*)	322 (*80.3*)	1 (*2.5*)	N/A	141 (*35.2*)	27 (*6.7*)	42 (*10.5*)	12 (*3.0*)	61 (*15.2*)	115 (*28.7*)

Table 2.

Specimens from 15–99 relative light unit (RLU) category using the *in vitro* diagnostic (IVD) Aptima Combo 2® (AC2) assay compared with results following retesting with the updated research-use only (RUO) Aptima Combo 2® assay.

IVD AC2 Assay			Qualitative Test Result Using RUO AC2 Assay (N [% of specimens])		
Qualitative Test Result	**RLU**	**No. of Specimens**	**Negative**	**Equivocal**	**Positive**
Negative for CT	15–32	16 *	13 (*81.3*)	0 (*0*)	3 (*18.8*)
	36–66	12 *†‡	6 (*50*)	0 (*0*)	6 (*50*)
	77–94	4 *	3 (*75*)	25 (*1*)	0 (*0*)
Equivocal for CT	32–48	8 *‡	2 (*25*)	0 (*0*)	6 (*75*)
	62–97	10 *†‡	3 (*30*)	0 (*0*)	7 (*70*)

*Specimen that failed sequencing

†Specimen with mutant 23S rRNA sequence

‡Specimen with wild-type 23S rRNA sequence