



Original Article

## Detecting *Ureaplasma urealyticum* among HIV-infected women with or without human papillomavirus using real-time PCR with the ANYPLEX™ II STI-7 assay system

John Taylor, MSc<sup>a,\*</sup>, Paul P. Sampene Ossei, MD<sup>b</sup>, Krishna Pradhan, MSc<sup>a</sup>,  
Joshua Adjah, PhD<sup>c</sup>, Eric Agyeman-Duah, MSc<sup>d</sup>, Bright O. Afranie, MPhil<sup>e</sup>,  
Sampson Donkor, MPhil<sup>e</sup> and William Ayibor, MPhil<sup>e</sup>

<sup>a</sup> Department of Molecular Biology and Biotechnology, Tezpur University, Assam, India

<sup>b</sup> Department of Pathology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology/Komfo Anokye Teaching Hospital, Kumasi, Ghana

<sup>c</sup> West Africa Center for Cell Biology of Infectious Pathogens (WACCBIP), Legon, Ghana

<sup>d</sup> Department of Molecular Medicine, Ulm University, Ulm, Baden-Württemberg, Germany

<sup>e</sup> Department of Molecular Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Received 15 December 2018; revised 2 April 2019; accepted 5 April 2019; Available online 8 May 2019

### المخلص

**أهداف البحث:** عدوى فيروس نقص المناعة البشرية يزيد فرص إصابة السيدات بفيروس الورم الحليمي البشري؛ العامل المسبب الرئيس لسرطان عنق الرحم. بالإضافة إلى ذلك، فإن من أكثر العوامل المرضية الشائعة المسببة للأمراض المنقولة جنسياً عند السيدات اللاتي لديهن فيروس نقص المناعة البشرية هي المَيُورَة الحالة لليوريا. تسعى هذه الدراسة لتحديد وجود المَيُورَة الحالة لليوريا بين السيدات اللاتي لديهن فيروس نقص المناعة البشرية مع وجود فيروس الورم الحليمي البشري أو عدمه في غانا.

**طرق البحث:** تم استخراج عينات الحمض النووي من مسحات عنق الرحم لـ 96 سيدة مصابة بفيروس نقص المناعة البشرية اللاتي حضرن لمستشفى كوماسي الجنوبي. وتم تشغيل نظام تفاعل البلمرة المتسلسل في الوقت الحالي على عينات الحمض النووي للكشف عن المَيُورَة الحالة لليوريا. استخدم مايكروسوفت اكسل لتحليل البيانات، واستخدم اختبار مربع كاي لتحديد العلاقات والتبعيات بين المتغيرات.

**النتائج:** بين المجموعة التي تم دراستها، 93.75% (96/90) كانت موجبة لمسبب مرضي واحد على الأقل؛ 36.5% (96/35) بالمَيُورَة الحالة لليوريا؛ و30.21% (96/29) سيدة كانت مصابة بالمَيُورَة الحالة لليوريا، واللواتي كن أيضاً مصابات بفيروس الورم الحليمي البشري و6.25% (96/6) كن سيدات مصابات بالمَيُورَة الحالة لليوريا وكن غير مصابات بفيروس الورم الحليمي البشري. لا يوجد ارتباط كبير (فاصل الثقة 95%) بين المَيُورَة الحالة لليوريا وفيروس الورم الحليمي البشري بين السيدات المصابات بفيروس نقص المناعة البشرية.

**الاستنتاجات:** المَيُورَة الحالة لليوريا وفيروس الورم الحليمي البشري هما مسببان للأمراض بدرجة عالية وانتشارهم في هذه الدراسة يكرر الحاجة إلى الفحص الروتيني للمريضات المصابات بفيروس نقص المناعة البشرية.

**الكلمات المفتاحية:** فيروس الورم الحليمي البشري؛ المَيُورَة الحالة لليوريا؛ فيروس نقص المناعة البشرية؛ مصاب بالعدوى؛ غانا

### Abstract

**Objectives:** Human immunodeficiency virus (HIV) infections increase the chances of women contracting human papillomavirus (HPV), the prime causative agent of cervical cancer. Additionally, *Ureaplasma urealyticum* is the most frequent pathogen prevailing in sexually transmitted diseases in HIV-infected women. The aim of this study was to determine the presence of *U. urealyticum* among HIV-infected women with or without HPV in Ghana.

**Methods:** DNA samples were extracted from cervico-vaginal swabs obtained from 96 HIV-infected women attending the Kumasi South Hospital. *U. urealyticum* in the DNA samples was detected by real-time PCR with the Anyplex™ II STI-7 detection assay. Microsoft Excel was used to analyze the data, and the Chi-square test was used to determine associations and dependence among the variables.

\* Corresponding address: Department of Molecular Biology and Biotechnology, Tezpur University, Assam, India.

E-mail: [ekowtaylor@yahoo.com](mailto:ekowtaylor@yahoo.com) (J. Taylor)

Peer review under responsibility of Taibah University.



Production and hosting by Elsevier

**Results:** Among the study population, 93.75% (90/96) were positive for at least one pathogen. In total, 36.5% (35/96) of the women were infected by *U. urealyticum*, with 30.21% (29/96) being co-infected with HPV. There was no significant association (95% CI,  $p > 0.05$ ) between *U. urealyticum* and HPV status among the HIV-infected women.

**Conclusion:** *U. urealyticum* and HPV are highly pathogenic, and their prevalence in this study reiterates the need for their routine screening in HIV-infected patients.

**Keywords:** Co-infection; Ghana; Human immunodeficiency virus; Human papillomavirus; *Ureaplasma urealyticum*

© 2019 The Authors.

Production and hosting by Elsevier Ltd on behalf of Taibah University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

The virulence of human papillomavirus (HPV) is greater among women infected with human immunodeficiency virus (HIV),<sup>1</sup> and higher rates of the infection have been shown to increase the risk for high-grade squamous intraepithelial lesions and cervical cancer, especially in women co-infected with HIV.<sup>2</sup> A study on the prevalence of sexually transmitted diseases in HIV-infected women revealed asymptomatic *Ureaplasma urealyticum* as being the most prevalent pathogen in approximately 30% of the study cohort.<sup>1</sup> Although the pervasiveness of HPV infections is not well established,<sup>3</sup> *U. urealyticum* is known to be persistent in 40–80% of sexually matured asymptomatic women and in approximately 25–40% of men.<sup>4</sup> The prevalence of *U. urealyticum* and its associated disorders is globally recognised as a serious public health concern. A number of research works have shown that non-gonococcal urethritis, miscarriage, neonatal pneumonia, preterm delivery, and chronic lung disease in preterm neonates are disorders associated with the *U. urealyticum* bacterium. Furthermore, HPV infections are known to cause invasive squamous cell carcinoma in either the anus or cervix,<sup>5</sup> and people co-infected with HIV are at a higher risk for cancer at either sites.<sup>6</sup> Studies suggest that this increased risk for cancer in individuals who have contracted HIV may be attributed to HIV-induced immune dysfunction or a longer persistence and increased replication of HPV.<sup>7</sup> In addition, an early investigation reported that the chances of a woman contracting HPV infections can be heightened by HIV infection, HIV-associated immunosuppression, or both.<sup>8</sup> In spite of the adverse simultaneous complications that may be presented by *U. urealyticum* infections among HIV-infected women with HPV, very little is known about the prevalence of such cases, especially in Ghana where there is currently little to no local studies in this regard. Therefore, this present study was carried out to determine the presence and prevalence of *U. urealyticum* among HIV-infected women with or without HPV and to establish the

association between *U. urealyticum* and HPV in HIV-infected women. The significance of this study was to provide reference data in a regional context on the pervasiveness of *U. urealyticum* among HIV-infected women with or without HPV. The molecular technique used in this study could also be adopted to optimise screening strategies for the detection of the microorganism and diagnosis of its infection.

## Materials and Methods

### Study design

This was a hospital-based study conducted at the Kumasi South Hospital, a regional hospital in the Kumasi metropolitan area, Ghana. The health facility serves as a referral hospital for other health facilities within and outside of Kumasi. It also runs a specialised HIV/AIDS clinic named the Chronic Care Unit, which also provides services for other chronic illnesses and antiretroviral therapy and has more than 4200 clients. All HIV-infected women (with an intact uterus) seeking health care at the clinic were eligible for the study. The patients were recruited regardless of their status as symptomatic or asymptomatic. All women reporting to the clinic were qualified for the study only after they had accepted to take part by signing a form of informed consent. In total, 96 out of 102 HIV-infected women agreed to take part in the study. From these women ( $n = 96$ ), cervico-vaginal swabs were taken, carefully packaged, and sent to the laboratory (Kumasi Centre for Collaborative Research) for analysis. The study was approved by the Committee on Human Research Publications and Ethics of the Kwame Nkrumah University of Science and Technology/Komfo Anokye Teaching Hospital, Kumasi, Ghana. Permission was also sought from the medical directors of the study facility.

### Laboratory tests

Seegene's Anyplex™ II STI-7 detection assay was used to detect *U. urealyticum* in the collected cervico-vaginal swabs. The Anyplex™ II STI-7 detection assay features a multiplex real-time PCR system which applies Seegene's accredited Dual Priming Oligonucleotide (DPO™) and Tagging Oligonucleotide Cleavage and Extension (TOCE™) technologies. The key constituents of TOCE™ are the DPO™ primer pairs, the pitcher and the catcher. The pitcher is a tagging oligonucleotide which hybridises specifically to the target region. The catcher is a fluorescence-labelled artificial template. The DPO™ primer and the pitcher hybridise specifically to the target sequence. During DPO™ primer extension, the pitcher is cleaved by the exonuclease activity of DNA polymerase, whereupon the tagging portion is released and hybridises to its complementary sequence on the catcher. Extension of the tagging portion separates the fluorescent reporter molecule from the quencher molecule, resulting in signal generation. The signal can be measured on a real-time basis and/or analysed by melting curve analysis to determine the presence of the target sequence. The Anyplex™ II STI-7 detection assay kit simultaneously (in a single real-time PCR) detects seven major sexually transmitted infection (STI)-causing pathogens with adequate sensitivity and specificity and at a cost similar to other kits, such as the Abbott kit which only

detects two pathogens at a time. The seven major STI-causing pathogens that can be detected by the Anyplex™ II STI-7 detection kit are *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *U. urealyticum*, and *Ureaplasma parvum*. For each sample, 5 µl of the extracted DNA was added to a final volume of PCR Master Mix consisting of 5 µl of 4X STI-7 TOM (amplification and detection reagents), 5 µl of 4X Anyplex PCR Master Mix (DNA polymerase, uracil-DNA glycosylase, and buffer containing dNTPs), and 5 µl of RNase-free water (ultrapure quality, PCR grade). One positive and one negative control were included in each run. The results after each run were presented as positives and negatives. The CFX96™ Real-Time PCR system (Bio-Rad) was set up to carry out the DNA amplification and detection. The real-time PCR was programmed according to the guiding procedures on the manufacturer's protocol and was examined with Seegene viewer software.

#### Statistical analysis

Microsoft Excel was used to analyze the data and to provide a graphical representation of the results. The Chi-square test was used to analyse the results for the relative risk of *U. urealyticum* infection among HIV-infected women.

## Results

#### Description of the study population

All the study participants (n = 96) were HIV-infected women within the age range of 22–57 years (average age 40.3 ± 3.5 years). The modal age ranges were 30–39 years

(41.67%) and 40–49 years (37.5%). Women between the ages of 50 and 59 years represented 14.58% of the total study population, and the least represented were women within the age range of 20–29 years (6.25%). Of all the study participants, 52% were married and all of them (n = 96) had children.

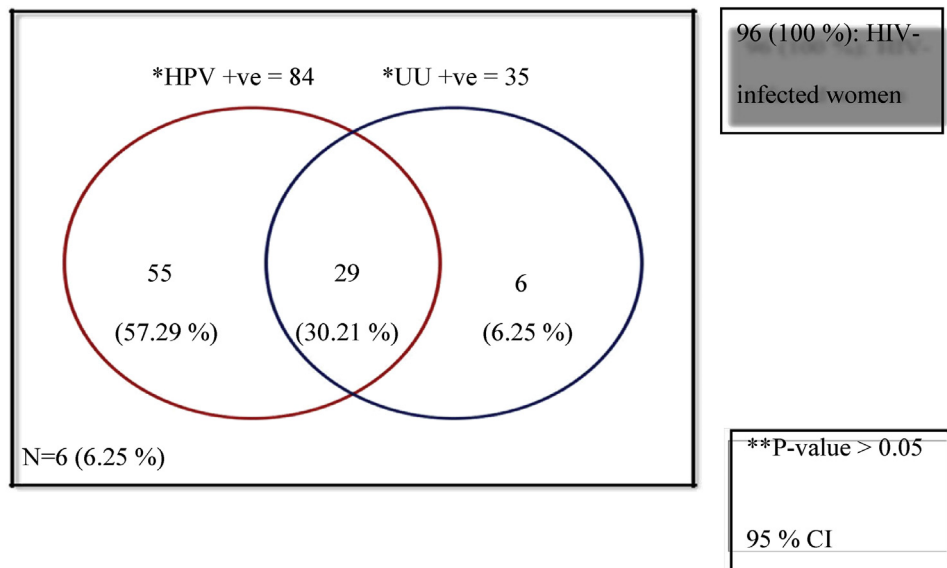
#### Description of Co-Infection status

Overall, 87.5% (84/96) of the study participants were positive for HPV. Results from the Anyplex™ II STI-7 detection assay showed that 41.6% (35/84) of those HPV-positive participants also tested positive for *U. urealyticum*. As shown in Fig. 1, 93.75% (90/96) of all study participants were positive for at least one of the pathogens (either HPV or *U. urealyticum*), whereas 6.25% (6/96) were not co-infected with any of these two pathogens. Among the study cohort, 63.54% (61/96) had co-infections. Moreover, 57.29% (55/96) of all participants were positive for HPV only and 6.25% (6/96) tested positive for *U. urealyticum* only. Overall, 30.21% (29/96) were positive for both pathogens.

#### Pathogen infection and age distribution

Of the 35 participants who tested positive for *U. urealyticum*, those within the age group of 40–49 years (51.43%; 18/35) had the highest prevalence of the bacterium, followed by those in the age group of 30–39 years (37.14%; 13/35). Participants within the ages of 20–29 and 50–59 years had the lowest infection rate (5.70% (2/35)). The results are illustrated in Table 1.

As shown in Table 2, most of the participants who were co-infected with both HPV and *U. urealyticum* were



\*HPV +ve: Human Papillomavirus – positive  
 \*UU +ve: *Ureaplasma urealyticum* – positive  
 \*\*P-value was 0.345 at 95% CI  
 N= HIV infected women with no other infections

**Figure 1:** Distribution of HPV and *U. urealyticum* infection among HIV-infected women.

**Table 1: Distribution of *U. urealyticum* among the study participants (n = 35/96) according to age.**

Age	UU (%)
20–29	2 (5.70)
30–39	13 (37.14)
40–49	18 (51.43)
50–59	2 (5.70)
Total	35 (100)

UU = *Ureaplasma urealyticum*.

**Table 2: Distribution of *U. urealyticum* and HPV co-infection according to age (n = 29/96).**

Age	UU/HPV Co-infection
20–29	2 (6.9)
30–39	12 (41.38)
40–49	12 (41.38)
50–59	3 (10.35)
Total	29 (100)

UU = *Ureaplasma urealyticum*, HPV = Human papillomavirus.

**Table 3: Distribution of *U. urealyticum* infection only (n = 6/35) according to age.**

Age	UU infected with no HPV Co-infection (%)
20–29	0 (0.0)
30–39	1 (16.67)
40–49	5 (83.33)
50–59	0 (0.0)
Total	6 (100)

UU = *Ureaplasma urealyticum*, HPV = Human papillomavirus.

between the ages of 30–49 years. As shown in Table 3, participants among the age groups of 30–39 and 40–49 years were predominantly infected with *U. urealyticum* but not HPV. None of the *U. urealyticum*-infected participants within the age groups of 20–29 and 50–59 years had HPV co-infection.

## Discussion

This study gives an account of the prevalence of *U. urealyticum* among HIV-infected women with or without HPV in Kumasi, Ghana and the use of real-time PCR with the Anyplex™ II STI-7 assay for the detection of the pathogen. Our PCR system demonstrated a high detection rate for *U. urealyticum* and was consistent with studies that also confirmed high detection rates for the same pathogen using the same molecular-based technique.<sup>1,9</sup> The results of our study indicated that 93.75% (90/96) of the HIV-infected women were co-infected with at least one of the pathogens (either HPV or *U. urealyticum*), whereas 6.25% (6/96) were not infected by any of the two pathogens. From Table 1, we observed that significantly more of the *U. urealyticum*-

infected women were aged 30 years or older; however, this finding contrasts with a study by Marovt and colleagues,<sup>10</sup> who reported that among 145 women, significantly more of the *U. urealyticum*-infected women were aged 25 years or younger. This inconsistency could be explained by the fact that in our study, a smaller fraction of the participants (6.25%; 6/96) was aged between 20 and 29 years, whereas the remaining 93.75% (90/96) of the women were aged 30 years or older. Clearly, *U. urealyticum* positivity could be skewed to women who were below 30 years with respect to our age distribution. From Fig. 1, we observed that there were approximately 5 times more *U. urealyticum*-positive women who were co-infected with HPV (30.21%; 29/96) than *U. urealyticum*-positive women who were not co-infected with the virus (6.25%; 6/96). This finding was essentially comparable to the results of a previous work which showed 6 times more *U. urealyticum*-positive women co-infected with HPV than those not co-infected with the virus.<sup>11</sup> The results of our work are consistent with those of other studies and suggest that the *U. urealyticum* infection rate is more frequent in HPV-positive women than in HPV-negative women.<sup>12,13</sup> Nonetheless, the P-value obtained after the Chi-square test indicated that there was no significant association ( $P > 0.05$ , 95% CI) between *U. urealyticum*-infected women who were co-infected with HPV and *U. urealyticum*-infected women who were not co-infected with HPV. The absence of significant association has some implications on the risks of HPV-infected and HPV-uninfected women in contracting *U. urealyticum*. This evidence from our study suggests that in HIV-infected women, the risk of contracting *U. urealyticum* is not dependent on whether the participants are HPV infected or not. In other words, HIV-infected women are liable to contracting *U. urealyticum* irrespective of their HPV status. However, the degree of association between some cervico-vaginal infections and HPV infections in other clinical studies has remained controversial and contentious, with some results pointing to the absence of association<sup>14</sup> and others proving a significant association between the two conditions.<sup>2</sup> We could not pinpoint this with accuracy as our investigation was subject to a number of limitations. Firstly, a lower number of the participants (6.25%; 6/96) were aged between 20 and 29 years, whereas a greater number of the women were between the age groups of 30–39 and 40–49 years. Evidently, the age distribution was not relatively proportional across the various age groups. Secondly, the distribution of the HIV-infected women living with HPV against those who were not living with the pathogen was not symmetrical, given that more than half of participants were HPV positive. Further investigations should be carried out on a larger sample size in order to provide a more definitive association between HPV and *U. urealyticum* co-infections. Findings from future work on a larger sample size – with careful consideration on the most affected age group(s) – could also add up to the findings of this study. It is suggested that for the HIV-infected participants, future studies should take into consideration a symmetrical ratio of HPV-infected and HPV-uninfected participants. Studies can also be carried out on women who are not living with HIV but have HPV and *U. urealyticum* co-infections, as such an investigation will allow us to measure the hostile reproductive complications associated with the co-infections in women.

## Conclusion

In conclusion, among our sample population of HIV-infected women, *U. urealyticum* was detected in both individuals with and those without HPV infection. There was no significant association ( $P > 0.05$ , 95% CI) between *U. urealyticum* infection among HPV-infected and HPV-uninfected women. This evidence therefore suggests that the risk of HIV-infected women in contracting *U. urealyticum* is not dependent on their HPV infectivity status. Real-time PCR with the Anyplex™ II STI-7 detection assay can rapidly detect microorganisms, especially *U. urealyticum*, and it is expected that this system will be used as a standard diagnostic test for STIs in the near future.

## Source of funding

No funding was obtained for this research.

## Conflict of interest

The authors have no conflict of interest to declare.

## Ethical approval

The study was approved by the Committee on Human Research Publications and Ethics (CHRPE) at the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, in collaboration with the management of Kumasi South Hospital. Participation was voluntary, and written informed consent was obtained from each participant according to the Helsinki Declaration. Respondents were assured that the information gathered was to be used strictly for research and academic purposes only. In addition, respondents were given the freedom to opt out at any time that they thought they could not continue with the study.

## Consent

As per international standards (Helsinki Declaration), written consent was collected from the participants and preserved by the authors.

## Authors' contributions

JT, PPSO, and JA conceived and designed the study, conducted research, provided research materials, and collected and organised the data. JT and JA analysed and interpreted the data. JT, JA, and BOA wrote the initial and final drafts of the article. KP, EAD, SD, and WA provided logistical support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

## Acknowledgment

The authors are grateful to the study participants and the staff of Kumasi South Hospital.

## References

1. Sylverken AA, Owusu-Dabo E, Yar DD, Salifu SP, Awua-Boateng NY, Amuasi JH, et al. Bacterial etiology of sexually transmitted infections at a STI clinic in Ghana; use of multiplex real time PCR. *Ghana Med J* 2016; 50(3): 142–148.
2. Moscicki A-B, Ellenberg JH, Farhat S, Xu J. Persistence of human papillomavirus infection in HIV-infected and-uninfected adolescent girls: risk factors and differences, by phylogenetic type. *J Infect Dis* 2004; 190(1): 37–45.
3. Jones M, Cook R. Intent to receive an HPV vaccine among university men and women and implications for vaccine administration. *J Am Coll Health* 2008; 57(1): 23–32.
4. Shahmanesh M, Moi H, Lassau F, Janier M. 2009 European guideline on the management of male non-gonococcal urethritis. *Int J STD AIDS* 2009; 20(7): 458–464.
5. Zbar A, Fenger C, Efron J, Beer-Gabel M, Wexner S. The pathology and molecular biology of anal intraepithelial neoplasia: comparisons with cervical and vulvar intraepithelial carcinoma. *Int J Colorectal Dis* 2002; 17(4): 203–215.
6. Madkan V, Cook-Norris R, Steadman M, Arora A, Mendoza N, Tyring S. The oncogenic potential of human papillomaviruses: a review on the role of host genetics and environmental cofactors. *Br J Dermatol* 2007; 157(2): 228–241.
7. Martin F, Bower M. Anal intraepithelial neoplasia in HIV positive people. *Sex Transm Infect* 2001; 77(5): 327–331.
8. Sun X-W, Kuhn L, Ellerbrock TV, Chiasson MA, Bush TJ, Wright Jr TC. Human papillomavirus infection in women infected with the human immunodeficiency virus. *N Engl J Med* 1997; 337(19): 1343–1349.
9. Choe H-S, Lee DS, Lee S-J, Hong S-H, Park DC, Lee M-K, et al. Performance of Anyplex™ II multiplex real-time PCR for the diagnosis of seven sexually transmitted infections: comparison with currently available methods. *Int J Infect Dis* 2013; 17(12): e1134–e1140.
10. Marovt M, Keše D, Kotar T, Kmet N, Miljković J, Šoba B, et al. *Ureaplasma parvum* and *Ureaplasma urealyticum* detected with the same frequency among women with and without symptoms of urogenital tract infection. *Eur J Clin Microbiol Infect Dis* 2015; 34(6): 1237–1245.
11. Camporiondo M, Farchi F, Ciccozzi M, Denaro A, Gallone D, Maracchioni F, et al. Detection of HPV and co-infecting pathogens in healthy Italian women by multiplex real-time PCR. *Infez Med* 2016; 24(1): 12–17.
12. Ji YI. Co-infections with human papillomavirus and mycoplasma/ureaplasma spp. in women with abnormal cervical cytology. *Res Rep Gynecol Obstet* 2017; 1(1).
13. Lukic A, Canzio C, Patella A, Giovagnoli M, Cipriani P, Frega A, et al. Determination of cervicovaginal microorganisms in women with abnormal cervical cytology: the role of *Ureaplasma urealyticum*. *Anticancer Res* 2006; 26(6C): 4843–4849.
14. Rahkola P, Mikkola TS, Ylikorkala O, Vaisanen-Tommiska M. Association between high risk papillomavirus DNA and nitric oxide release in the human uterine cervix. *Gynecol Oncol* 2009; 114(2): 323–326.

**How to cite this article:** Taylor J, Sampene Ossei PP, Pradhan K, Adjah J, Agyeman-Duah E, Afranie BO, Donkor S, Ayibor W. Detecting *Ureaplasma urealyticum* among HIV-infected women with or without human papillomavirus using real-time PCR with the ANYPLEX™ II STI-7 assay system. *J Taibah Univ Med Sc* 2019;14(3):295–299.