Characteristics of the Sample Adequacy Control (SAC) in the Cepheid Xpert® CT/NG Assay in Female Urine Specimens

Abstract

Background: The Xpert® CT/NG (Cepheid Sunnyvale, CA) is a rapid, fully automated real-time polymerase chain reaction test that simultaneously detects Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG). It has high sensitivity and specificity, but also includes a Specimen Adequacy Control (SAC). SAC controls for false negative results by confirming adequate patient sample and appropriate testing conditions. SAC is quantified by its cycle threshold (Ct), the number of cycles required to detect the presence of a single copy human gene. A lower SAC indicates an earlier Ct, and more human cellular material detected. Our objectives were to describe the frequency and distribution of SAC Ct values and observe any correlations with detected infections.

Methods: Urine samples from 1382 HIV-1-infected pregnant women, collected at the time of labor/delivery underwent Xpert® CT/NG testing. Mean SAC Ct values and standard deviation (SD) were calculated. Student’s t-test was used to compare mean SAC Ct values to a reference of urine samples negative for CT and NG.

Results: The urine CT positivity was 17.9% (248/1382) and NG, 4.6% (63/1382). The mean SAC Ct value in urine from women without CT or NG was 28.09 (SD: 4.12) and higher than the mean SAC Ct value for CT positive specimens (27.29, SD: 3.84(P<.0054)), NG positive specimens (26.23, SD: 3.09(P<.0001)), and specimens positive for both CT and NG (26.41, SD: 3.01(P=.0027)).

Conclusion: Lower SAC Ct values were significantly associated with chlamydial and gonococcal infections. Further studies should be conducted to determine the utility of SAC Ct values for identifying the presence of increased human cellular material and infection.

Keywords
Sample adequacy control; Chlamydia; Gonorrhea; Diagnosis; Inflammation

Abbreviations

HIV: Human Immunodeficiency Virus; ART: Anti-Retroviral Therapy; NICHD: National Institute of Child Health and Human Development; CDC: Center for Disease Control; HPTN: HIV Prevention Trial Network; STI: Sexually Transmitted Infection; CT: Chlamydia trachomatis; NG: Neisseria gonorrhoeae; Ct: Cycle Threshold; SAC: Specimen Adequacy Control; SD: Standard Deviation; PCR: Polymerase Chain Reaction; HMBS: Hydroxymethylbilane Synthase; SPC: Sample Processing Control; PCC: Probe Check Control; FDA: Food and Drug Administration

Summary

Xpert® CT/NG, a rapid, automated real-time PCR assay, includes a Specimen Adequacy Control (SAC). A secondary use of the SAC cycle threshold values may be as a marker for inflammation.

Introduction

Sexually transmitted infections (STIs) of Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) continue to place an immense health burden on women worldwide. CT and NG infections are the most common STIs and accounted for 211.8 million cases globally in 2008 [1]. In the United States in 2009 alone, over 1.2 million chlamydia infections were reported to the U.S. Center for Disease Control (CDC) making it the most commonly reported notifiable disease [2,3]. Gonorrhea is the second most commonly reported notifiable disease in the United States with over 300,000 cases reported in 2009.

The immunologic response triggered by lower genital tract infections with Chlamydia trachomatis and Neisseria gonorrhoeae leads to significant inflammation of the cervico-endometrial tissue [4]. Due to infection and the associated inflammatory response, several important sequelae may result from these conditions, including pelvic inflammatory disease, ectopic pregnancy, and infertility [3-6]. Routine screening of STIs is recommended for young women and at-risk groups including pregnant women because of the frequency of infection and the adverse outcomes associated with untreated infections.

The Cepheid Xpert® CT/NG (Cepheid Sunnyvale, CA) is a point-of-care fully automated real-time polymerase chain reaction (PCR) test. It is a rapid molecular assay for simultaneous
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Adequacy Control (SAC) set

Materials and Methods

Patient population

Specimen collection

Citation: Bristow CC, Adachi K, Nielsen-Saines K, Ank B, Morgado MG, et al. (2014) Characteristics of the Sample Adequacy Control (SAC) in the Cepheid Xpert® CT/NG Assay in Female Urine Specimens. J Microbiol Exp 1(4): 00026. DOI: 10.15406/jmen.2014.01.00026
2/3/2014.

Results

Urine samples from 1406 HIV-1-infected women were tested using the Xpert® CT/NG assay on the GeneXpert platform. Of the 1406 specimens, 47 (3.3%) had indeterminate results. Those samples were rerun up to two times, and 23 of the 47 samples retested gave a valid result. The 24 (1.7%) samples that continued to give indeterminate results were excluded from analysis. Amongst the 1382 samples remaining after exclusion of invalid/error results, results were positive for CT in 248 (17.9%). NG infection was detected in 63 (4.6%) of the samples. Thirty-five (2.5%) of specimens were positive for both CT and NG.

The mean SAC C value in urine from women not infected with CT or NG was 28.09 (SD: 4.12), this was used as the reference for bivariate comparisons. This reference cycle threshold value was higher than the mean SAC C value for CT positive specimens, NG positive specimens and those co-infected with both CT and NG. The SAC C value of specimens from women infected with CT was 27.29 (SD: 3.84, P=0.0054). For specimens from women infected with NG, the SAC C value was 26.23 (SD: 3.09, P<0.0001). Amongst the specimens from women co-infected with both CT and NG, the SAC C value was 26.41 (SD: 3.01, P=0.0027) (Table 1, Figure 1).

Specimens from mothers who transmitted HIV infection to their infants had a mean SAC C value of 27.81 (SD: 4.06). This value was not significantly different from that of specimens obtained from women uninfected with NG and CT who did not transmit HIV to their infants, mean SAC C value of 28.11 (SD: 4.12, p= 0.4517) (Table 2).

Discussion

This study investigated distribution of the SAC C, a quality control mechanism in the Xpert® CT/NG assay that reports the number of replication cycles required to reach a detection threshold for the control HMBS gene. We found that lower SAC C values were associated with CT and NG infections in urine samples collected from HIV-1-infected women enrolled in NICHD HPTN 040. Each cycle in the PCR process represents doubling the amount of target DNA, this is a two fold increase each time the C increases by a value of 1. As each cycle occurs, the amount of DNA will increase exponentially. Therefore a difference in the C that may seem small is in fact a very substantial difference in the absolute amount of DNA target. This association of lower SAC C values with CT and NG infections was of statistical significance in this patient population recruited from Brazil, South Africa, Argentina and the United States.

Our findings that SAC C values in CT and/or NG infected women were lower than those observed in urines of CT and NG uninfected women is suggestive of increased amounts of human cellular material in these urine samples.

Most CT and NG infections are asymptomatic [5] and little is known about the risk of consequences and transmission of asymptomatic infections. The SAC C may reflect cell turnover, burden of disease and inflammation and therefore should be evaluated clinically with particular focus on the risk for adverse clinical sequelae in asymptomatic patients. The degree to which the SAC C value correlates with the burden or severity of infection is unknown at this time and may represent a potential area for future research. If the SAC C value is associated with bacterial burden or inflammatory response to infection, this may have implications for predicting the likelihood for adverse sequelae, perinatal transmission, and adverse birth outcomes associated with genital infections like CT and NG. Longitudinal studies are needed to look at associations between SAC C values, measures of infection, and clinical outcomes.

This study was subject to some limitations. First, the study population was comprised exclusively of HIV-1-infected pregnant women who were potentially immunocompromised and could have altered inflammatory responses to CT and NG infection, however, most women enrolled in the parent 040 study had asymptomatic HIV infection and CD4 cell counts over 400 cells/mm³. Another limitation is that women in labor or recently delivered may have increased human cellular material in urine because labor is associated with a substantial inflammatory burden or inflammatory response to infection.

Table 1: Sample adequacy control (SAC) cycle threshold (C) values for urine samples from HIV-1-infected pregnant women by infection status.

|                  | N  | Mean | Std | Median | IQR | Max | Min | p-value |
|------------------|----|------|-----|--------|-----|-----|-----|---------|
| CT+              | 248| 27.29| 3.84| 27.05  | 4.40| 38.90| 17.70| 0.0054  |
| NG+              | 63 | 26.23| 3.09| 26.20  | 3.60| 32.10| 17.70| <0.0001 |
| Co-infected, NG+ and CT+ | 35 | 26.41| 3.01| 25.90  | 3.90| 32.10| 17.70| 0.0027  |
| At least one infection, NG+ or CT+ | 276| 27.16| 3.80| 27.05  | 4.35| 38.90| 17.70| 0.0007  |
| Uninfected with CT and NG | 1106| 28.09| 4.12| 27.60  | 5.60| 43.40| 18.70| REF *
| TOTAL            | 1382| 27.90| 4.07| 27.50  | 5.40| 43.40| 17.70|         |

P-values were generated using student’s t-test comparing SAC C mean values for infection categories to the SAC C reference shown.

Table 2: Sample adequacy control (SAC) cycle threshold (C) values for urine samples from HIV-1-infected pregnant women by mother-to-child HIV transmission status.

|                  | N  | Mean | Std | Median | IQR | Max | Min | p-value |
|------------------|----|------|-----|--------|-----|-----|-----|---------|
| HIV acquisition in infant | 119| 27.81| 4.06| 27.60  | 5.50| 39.10| 20.30| 0.5522  |
| No HIV acquisition in infant and uninfected with CT and NG | 1015| 28.11| 4.12| 27.60  | 5.70| 43.40| 18.70| REF * |

P-values were generated using student’s t-test comparing SAC C mean values for infection categories to the SAC C reference shown.

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response. Nevertheless, in spite of a recent labor and a potentially immune compromised population, we still found SAC Ct values to be significantly associated with NG and CT infection status. Our study was cross sectional and thus had no follow up information for the women or infants, but sets the stage for future such studies.

The association between SAC Ct values and detection of lower genital tract infections is promising for use with urine samples, but the performance of the SAC will also need to be evaluated using other commonly collected specimen types like vaginal swabs. While further studies are needed, SAC Ct values may show early promise as a marker of clinical diagnosis of genital tract infections such as CT and NG.

Acknowledgment

The NICHD HPTN 040 study was supported by NICHD Contract # HHSN267200800001C, N01-HD-8-0001 and U01 AI047986 (Brazilian AIDS Prevention Trials International Network), NIAID/NIH. Overall support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) was provided by the National Institute of Allergy and Infectious Diseases (NIAID) [U01 AI068632], the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and the National Institute of Mental Health (NIMH) [AI068632]. In addition, the study was supported in part by Boehringer Ingelheim Pharmaceuticals Inc. (BPI), and GlaxoSmithKline on behalf of ViVi HealthCare. The authors would like to acknowledge the assistance of Ms. Mary Ann Hausner and Ms. Jessica Liu who worked diligently in the laboratory in the specimen preparation for the urinary analyses. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Cepheid donated all testing supplies and conducted testing for urine specimen analysis for the detection of infections of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

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