Y-Chromosome Detection among African American Women Who Have Sex with Women (WSW)

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Background

Sexual behaviors are prone to reporting bias. Use of biomarkers of semen exposure such as Y-chromosome detection in vaginal fluid are one approach to reduce this bias (Snead et al 2014). This is of particular interest when evaluating WSW for female-to-female transmission of sexually transmitted infections (STIs) as current/recent sex with male sexual partners could confound the situation.

One study found detection of vaginal Y-chromosome sequences up to 15 days following unprotected penile-vaginal intercourse with a mean clearance half-life of 3.83 days and a lower detection limit of 5 chromosome copies (Zenilman et al 2005). Condom use during intercourse has appeared to prevent vaginal Y-chromosome detection (Ghanem et al 2007). However, the sensitivity for detecting Y-chromosome in the setting of oral sex or digital penetration in the preceding 48 hours has been found to be low at 19% and 27%, respectively (Ghanem et al 2007).

To date, the majority of studies performing Y-chromosome testing have occurred in heterosexual women. No studies have performed this testing on vaginal specimens collected from women who have sex with women (WSW) who denied recent sexual activity with men.

Objective

The objective of this study was to perform Y-chromosome testing on vaginal specimens from a cohort of exclusive African American WSW who denied recent sex with men in the past 30 days to determine the accuracy of their self-reported sexual behaviors.

Methods

Sample Collection: Vaginal specimens stored at -80ºC in 10% AssayAssure from 78 WSW presenting to an urban STI clinic and participating in a study of partnership characteristics and STIs (Muzny et al., 2014) were used for this study. All women denied recent sexual activity with men during the past 30 days.

Sample Preparation: 100 µL of vaginal specimen wash was treated overnight with Ready-Lyse lysozyme (EpiCentre Technologies, Madison, WI) at 37ºC, followed by 1 hour at 56ºC with Proteinase K and DTT in Qiagen Buffer ATL (Dneasy Blood and Tissue kit, Qiagen Inc., Valencia, CA). Standard column based extraction was performed.

Amplification: Amplification was carried out using the Quantifiler Y Human Male DNA Quantification kit (Life Technologies, Carlsbad, CA). Amplification conditions were as follows: (95ºC x 10') x 1/ (95ºC x 15"; 60ºC x 60") x 50, with a 25 µL reaction volume using 2 µL of template DNA.

Analysis: The run reaction set included an 8-point 3-fold dilution series (50 ng/µL to 23 pg/µL) using the kit's supplied standard. The Cq values were determined by 2nd derivative maximum analysis using the LightCycler software with known positive and negative controls. The standard curve efficiency is 2.084 (Error 0.0219). The limit of detection was set at 5-times the standard deviation of the lowest measurable standard, 36 pg/µL (28 pg/µL + 5X-STD 1.44 pg/µL), or approximately 11 haploid sperm cells.

Results

Table 1 shows select demographic, sexual history, and current STI diagnoses for this cohort of women.

Table 1. Selected Demographic and Sexual History Characteristics of African American WSW Presenting to the Jefferson County Department of Health STI Clinic, August 2011 – October 2013

	African American WSW (n = 78)
Age (mean, SD)	26.3 (8.5)
Has high school degree	67 (85.9%)
Has primary care provider	36 (46.1%)
Has health insurance	43 (55.1%)
Alcohol use, past 30 days	56 (71.8%)
Tobacco use, past 30 days	47 (61.0%)
Illicit drug use, past 30 days	25 (32.0%)
History of STIs	41 (52.6%)
Current STI diagnosis	
CT (NAAT)	2 (2.6%)
GC (NAAT)	0
TV (wet mount and Inpouch™culture)	13 (16.7%)
HSV-2 Serology	20 (25.6%)

STI = sexually transmitted infection; CT = *Chlamydia trachomatis*; GC = *Neisseria gonorrhoeae*; TV = *Trichomonas vaginalis*; HSV-2 = Herpes Simplex Virus Type 2; NAAT = nucleic acid amplification test

Three participants were initially determined to be positive for Y-chromosome; 2 were later found to be false positives: (1) WSPH-163 which had a Y-chromosome concentration of 15 pg/µL (below the standard curve LOD of 36 pg/µL) and (2) WSPH-100 (selected by the software in error based on borderline noise threshold; after resetting the noise threshold, WSHP-100 was no longer positive).

WSPH-045 tested as a true positive for Y-chromosome within the boundaries of the standard curve **(Figure 1).** This participant's sample contained 230 pg/µL of template Y-chromosomal DNA or approximately 62 sperm/µL.

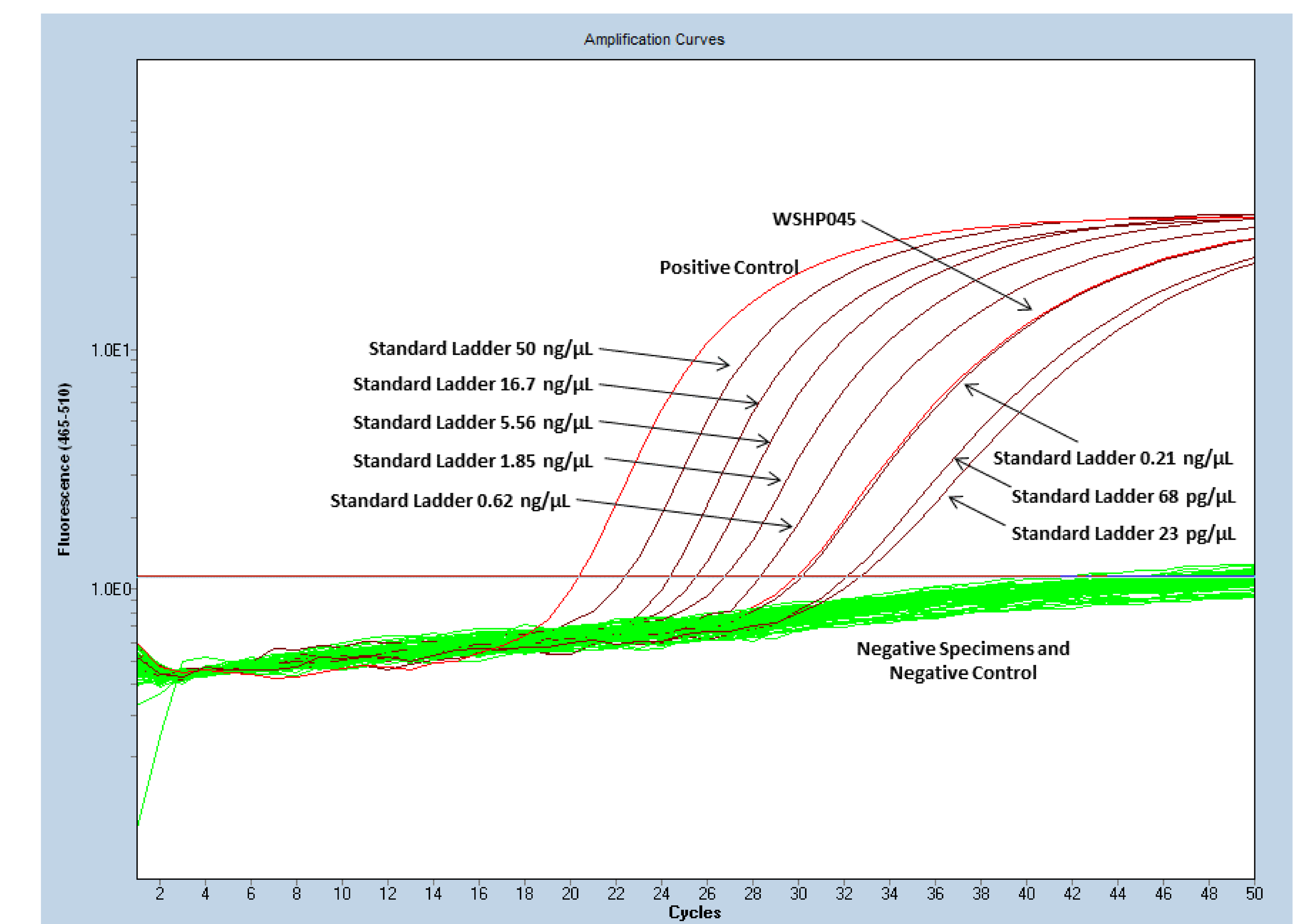

Figure 1. Y-Chromosome qPCR Amplification Curves. Cycle number (x-axis) plotted against reporter fluorescence (FAM, 465-510 nm, y-axis); the true positive specimen is bright red, negative specimens are green, and standards are brown. The positive control was known male DNA; the negative control (a negative template control) is within the green curves.

Conclusions

The very small proportion (1/78; 1.3%) of African American WSW with a Y-chromosome detected in their vaginal fluid suggests that these women accurately reported their recent sexual behaviors with men. These data are limited in that Y-chromosome can only be detected up to 15 days following unprotected penile-vaginal intercourse. Nevertheless, Y-chromosome testing may be useful in future WSW STI studies.

References

References available upon request.