JCM Accepts, published online ahead of print on 13 August 2014
J. Clin. Microbiol. doi:10.1128/JCM.01614-14

Comparison of Flocked and Aptima Swabs and Two Specimen Transport Media in the Aptima Combo 2 Assay

Running Title: AC2 Testing of Swabs and Transport Media

Jenny Li[1], Dan Jang[1], Jodi Gilchrist[1], Marek Smieja[1], Ruth Ewert[2], Cindy MacRitchie[3], and Max Chernesky[1*]

[1]St. Joseph's Healthcare/McMaster University, Hamilton, ON, CAN
[2]Evergreen Health Centre, Toronto, ON, CA
[3]Hamilton Community Health Centre, Hamilton, ON, CAN

*Corresponding Author:
Max Chernesky
St. Joseph's Healthcare
50 Charlton Ave E.
Hamilton, ON
L8N 4A6
chernesk@mcmaster.ca

905-522-1155 (ext. 35012)

Word count: Abstract 50 (max 50words); text 1045 (max 1000 words)

ABSTRACT

Self-collected vaginal Aptima swabs and flocked swabs in Aptima specimen transport media and ESwabs in ESwab media detected all 37 *C. trachomatis* infected patients from 287 women tested by the Aptima Combo assay (n=287). Prevalence rates of *C. trachomatis*, *N. gonorrhoeae*, and dual infection were 12.8%, 3.1% and 2.4%, respectively.

The Aptima Combo 2 (AC2) transcription-mediated amplification assay (Hologic|Gen-Probe, San Diego, CA) has been shown to be effective for testing first void urines and vaginal swabs (1,2). A novel nylon flocked swab has been developed by Copan Italia and was shown to enhance the analytical sensitivity of AC2, AMPLICOR (Roche, Basel, Switzerland) and ProbeTec (Becton Dickinson, Franklin Lakes, NJ) nucleic acid amplification tests (NAAT) for *C. trachomatis* and *N. gonorrhoeae* in mocked samples (3). It is thought that enhancement is accomplished by stripping more cells during collection and releasing more analyte into the transport media for testing (4). As diagnostic laboratories expand their testing menus for microorganism recovery by growth, antigen detection, NAATs and sequencing, the ability to universally collect, transport and test with crossover compatibility of assays becomes desirable (5-7). The study objectives were to compare AC2 testing of combinations of vaginal swabs and transportation media and first void urine.

A total of 287 women signed consent for self-collection of a first void urine (first 10-20 ml) and the collection of randomized vaginal swabs collected as follows: (a) Aptima Vaginal Swab (Hologic|Gen-Probe, Cat. No. 301162) in Aptima specimen transport media; (b) a regular flocked swab (Copan Italia, Brescia, Italy, Cat. No. 519CS01) transported in Aptima specimen transport media; (c) Copan ESwab Collection Kit (Copan Italia, Cat. No. 480CE) containing a regular flocked ESwab transported in ESwab media. The Aptima swab has Dacron fibers wrapped around the end of a plastic shaft and is cleared for use in the AC2 assay following transportation in Aptima specimen transport media. The flocked swab has short nylon fibers glued to the end of a plastic shaft and has been used as a collection device for the diagnosis of many infections by NAAT (8,9). The ESwab is a flocked swab used with ESwab media for subsequent recovery of organisms by culture (5,6). For self-collection of

vaginal swabs, the plastic container was opened to take out a swab and vial. The swab was held at a mark on the swab shaft and inserted, so the fingertips were just inside the vulva. The swab was rotated in a circular fashion to brush against the vaginal wall. After 5 turns the swab was placed into the transport tube, the shaft broken and tube capped then sent to the laboratory. The Aptima and flocked swabs were kept in the Aptima specimen tubes and tested by AC2 as recommended in the manufacturer's package insert. The ESwabs were removed without agitation from the ESwab media and placed into an Aptima tube for testing. First void urine (1mL) was aliquoted from the specimen containers into Aptima urine transport tubes. All three swabs and first void urine were simultaneously tested within 24 hours with the AC2 test on the Tigris (Hologic|Gen-Probe).

Calculations of sensitivity, specificity and predictive values with confidence intervals were made with 2 x 2 tables. Women were considered infected if 2 or more of the samples were positive.

The prevalence was 12.9% (37/287) for *C. trachomatis*, 3.1% (9/287) for *N. gonorrhoeae* and included 7 (2.4%) with dual infections. From study patient forms, 34.8% of the women reported symptoms of discharge, dysuria or pelvic pain, with no rate differences in infected or uninfected women. Table 1 summarizes the sensitivity, specificity and predictive values according to the self-collected vaginal swab and the transport system used and the first void urine sample. All *C. trachomatis* positive patients (37/37) were detected by the Aptima swab and flocked swab in Aptima specimen transport media and the ESwab in ESwab media. Five cases were only positive for *C. trachomatis* in a single swab type (3 Aptima swabs in Aptima specimen transport media and two flocked swabs in Aptima specimen transport media). On repeat testing, one flocked swab and three Aptima swabs repeated

positive, suggesting that they may have been true positives. However, confirmatory testing with an additional assay, using alternate primers, was not performed due to insufficient sample volume. The sensitivity of *C.* trachomatis on first void urine was 100% (34/34), which was higher than reported in previous studies (1,2) and may have been due to increased accuracy in collecting first void urine in this group of women, or not having a cervical swab result to broaden the reference standard. Although the number of *N.* gonorrhoeae positives were few (n=9), all of the sampling and transportation strategies identified 100% of the positives and negatives except for the ESwab in ESwab media, which missed one positive. Shortcomings of the study were the limited number of *N. gonorrhoeae* positives and our lack of attempting the ability to culture *N. gonorrhoeae* or *C. trachomatis*. Further studies of culturing *N. gonorrhoeae* NAAT-positives from the transportation vial would facilitate antibiotic resistance studies. Van Horn has shown successful recovery of gram-positive and negative bacteria from the ESwab system (5,6) but Indevuyst et al. (7) reported ESwabs to be toxic for cell cultures used to isolate viruses. More detailed controlled studies are required.

Specimens processed within 24 hrs of collection may have favoured the ESwab media to yield results equal to Aptima specimen transport media. Longer periods of holding time need to be studied to determine whether ESwab media provides enough stability for rRNA detection in AC2. Le Roy et al. used flocked swabs transported in culture media to study agreement of *C. trachomatis* positivity between cobas 4800 and cobas TaqMan (10), but they did not compare the off-label use of the flocked swab with the Roche collection and transportation kit. Although information from these kinds of studies may facilitate diagnosis, off-label use may provide limitations and require appropriate validation.

A previous laboratory study with mocked samples (3) compared kit swabs to flocked swabs and showed an enhancement of the endpoint of detection (analytical sensitivity) by flocked swabs in AC2, AMPLICOR, and ProbeTec assays. The phenomenon was not observed on clinical specimens using AC2 in this study as both swab types and transport media yielded maximum numbers of positives. Similar observations were made comparing vaginal Dacron swabs to flocked swabs put into Aptima specimen transport media for detection of *Trichomonas vaginalis* using analyte specific reagents for the Aptima assay (11). Ease of collection, performance and cost efficiency should be considered when studies comparing collection and transportation of clinical samples are compared in other NAATs to determine universality.

Acknowledgements

This study was funded by Copan Italia.

This publication is dedicated to the late Daniele Triva.

REFERENCES

1. Schachter J., Chernesky M., Willis DE., Fine PM., Martin DH., Fuller D., Jordan JA., Janda W., Hook III EW. Vaginal swabs are the specimens of choice when screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: results from a multicenter evaluation of the APTIMA assays for both infections. Sex Transm Dis. 2005. 32[12]:725-728.
2. Chernesky M., Jang D., Luinstra K., Chong S., Smieja M., Cai W., Hayhoe B., Portillo E., MacRitchie C., Main C., Ewart R. High analytical sensitivity and low rates of inhibition may contribute to detection of *Chlamydia trachomatis* in significantly more women by the APTIMA combo 2 assay. J. Clin Microbiol. 2006. 44[2]:400-405.
3. Chernesky M., Castriciano S., Jang D., Smieja M. Use of flocked swabs and a universal transport medium to enhance molecular detection of *Chlamydia trachomatis* and *neisseria gonorrhoeae*. J. Clin. Microbiol. 2006. 44[3]:1084-1086.
4. Daley P., Castriciano S., Chernesky M., Smieja M. Comparison of flocked and rayon swabs for collection of respiratory epithelial cells from uninfected volunteers and symptomatic patients. J. Clin. Microbiol. 2006. 44:2265-2267.
5. Van Horn KG., Audette CD., Sebeck D., Tucker KA. Comparison of the Copan eSwab system with Two Amies agar swab transport systems for maintenance of microorganism viability. J. Clin. Microbiol. 2008. 46[5]:1655-1658.
6. Van Horn KG., Audette CD., Tucker KA., Sebeck D. Comparison of 3 swab transport systems for direct release and recovery of aerobic and anaerobic bacteria. Diagn. Microbiol. Infect. Dis. 2008. 62:471-473.
7. Indevuyst C., Beuselinck K., Lagrou K. eSwab flocked swabs unfit for viral culture. J. Clin. Virol. 2012. 55:282-283.
8. Krech T., Castriciano S., Jang D., Smieja M., Enders G., Chernesky M. Detection of high risk HPV and *Chlamydia trachomatis* in vaginal and cervical samples collected with flocked nylon and wrapped rayon dual swabs transported in dry tubes. J. Virol. Methods. 2009. 162:291-293.
9. Lawson L., Lytwyn A., Jang D., Howard M., Elit L., Onuma K., Klingel M., Toor R., Gilchrist J., Ecobichon-Morris A., Smieja M., Chernesky M. Comparison of APTIMA HPV E6/E7 mRNA and Hybrid Capture 2 Assays using Wet and Dry Self-Collected Flocked Vaginal Swabs and PreservCyt L-Pap Samples. J. Anal. Oncol. 2012. 1(2):149-154.
10. Le Roy C., Papaxanthos A., Liesenfeld O., Mehats V., Clerc M., Bebear C., de Barbeyrac B. Swabs (dry or collected in universal transport medium) and semen can be used for the detection of Chlamydia trachomatis using the cobas 4800 system. J. Med. Microbiol. 2013. 62:217-222.
11. Jang D., Gilchrist J., Portillo E., Smieja M., Toor R., Chernesky M. Comparison of Dacron and nylon-flocked self-collected vaginal swabs and urine for the detection of Trichomonas vaginalis using analyte-specific reagents in a transcription-mediated amplification assay. Sex Transm. Infect. 2012. 88(2):160-162.

Table 1: Sensitivities, Specificities and Predictive Values of Self-Collected Vaginal Aptima Swabs and Flocked Swabs Transported in Aptima Specimen Transport Media (ASTM) or E-swab Media (ESM) Compared to First Void Urine by Aptima Combo 2 Testing for *C. trachomatis*

			Predictive Values	
Sample and Transport	**Sensitivity (%, CI)**	**Specificity (%, CI)**	**Positive (%, CI)**	**Negative (%, CI)**
Aptima swab in ASTM	37/37 (100, 88.8-100)	247/250 (98.8, 96.4-99.8)	37/40 (92.5, 79.4-98.1)	247/247 (100, 98.2-100)
Flocked swab in ASTM	37/37 (100, 88.8-100)	249/250 (99.6, 97.6-100)	37/38 (97.4, 85.3-100)	249/249 (100, 98.2-100)
E-swab in ESM	37/37 (100, 88.8-100)	250/250 (100, 98.2-100)	37/37 (100, 88.8-100)	250/250 (100, 98.2-100)
First Void Urine	34/34 (100, 98.2-100)	220/220 (100, 98.2-100)	34/34 (100, 88.2-100)	220/220 (100, 98.2-100)

*CI – 95% confidence intervals

For *N. gonorrhoeae* all samples showed 100% specificity (278/278) and 100% sensitivity (9/9), except for E-swab in ESM which showed 88.9% sensitivity (8/9).