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Identifying a consensus sample type to test for *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Trichomonas vaginalis* and human papillomavirus

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1 INTENDED CATEGORY

2 Original article

3 TITLE

- 4 Identifying a consensus sample type to test for Chlamydia trachomatis, Neisseria
- 5 gonorrhoeae, Mycoplasma genitalium, Trichomonas vaginalis and human papillomavirus

6 RUNNING TITLE

7 The optimal sample type to diagnose STIs

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25 ABSTRACT

Objectives: Sexually transmitted infections (STIs) are a global cause of acute illness. Early 26 detection plays a crucial role in interrupting transmission and preventing complications. 27 However, the accessibility of STI testing is curbed by the lack of an overall preferred sample 28 type. By means of a prospective study in female sex workers (FSW), we compared the 29 sensitivity of samples from different anatomical sites in detecting *Neisseria gonorrhoeae*, 30 Chlamydia trachomatis, Trichomonas vaginalis, Mycoplasma genitalium and human 31 papillomavirus. Besides, we documented the prevalence of each STI in this high-risk 32 33 population.

Methods: We selected 303 FSW and tested them for each STI by nucleic acid amplification testing on two vaginal and cervical swabs from different manufacturers, cervical smearand first-void urine. The sensitivity of each sample type was compared for each infectious agent in order to identify a consensus sample type.

Results: Vaginal swabs were superior to all other sample types, with an overall sensitivity of
86%. The sensitivity was the lowest for first-void urine, detecting only 63% of positive cases.
The prevalence was 3.3% (10/299) for *Neisseria gonorrhoeae*; 9.0% (27/299) for *Chlamydia trachomatis*; 7.4% (22/298) for *Trichomonas vaginalis*; 10.8% (32/296) for *Mycoplasma genitalium* and 55.6% (158/284) for human papillomavirus.

43 Conclusions: When testing for STIs, vaginal swabs are the sample of choice and first-void
44 urine should be avoided. Designating (self-sampled) vaginal swabs as a consensus sample
45 type enables harmonization of STI testing and extension of testing to large numbers of
46 unscreened females.

48 KEYWORDS

- 49 Sexually transmitted infections
- 50 Laboratory diagnosis
- 51 Genital samples
- 52 Chlamydia trachomatis
- 53 Neisseria gonorrhoeae
- 54 Human papillomavirus
- 55 Mycoplasma genitalium
- 56 Trichomonas vaginalis

58 INTRODUCTION

Sexually transmitted infections (STIs) are a main global cause of acute illness, leading to 59 60 serious complications, e.g. infertility. Early detection of (a)symptomatic infections has a 61 crucial role in lowering the prevalence and hence preventing complications. Multiple molecular diagnostic tools have been developed to detect a variety of STIs but consensus on 62 an overall preferred sample type lacks. Harmonization of testing is needed in order to 63 improve the accessibility, in particular for hard-to-reach populations. This will result in 64 diagnosis and treatment available more quickly in a patient-friendly way, thereby curbing 65 the clinical impact of STIs. 66

67

To encourage this harmonization, we searched for a consensus sample type to test for the 68 five most prevalent STIs for which nucleic acid amplification testing (NAAT) is the preferred 69 70 diagnostic tool: Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, 71 Mycoplasma genitalium and human papillomavirus (HPV). We compared the sensitivities of six sample types (two vaginal and cervical swabs from different manufacturers, a cervical 72 73 smear and a first-void urine) in detecting each STI. By documenting the prevalence of (co-74)infections, data were collected on the local epidemiology and insight was gained in the clinical relevance and need for simultaneous detection. 75

76 MATERIALS AND METHODS

Between June 2015 and June 2016, a prospective study was conducted, including 303 female sex workers (FSW) with a mean age of 33 years (range 18-58 years), embedded in a health program for sex workers in Flanders (PASOP). PASOP provides specific outreach occupational health services, focused on prevention e.g. STI screening, vaccination against hepatitis B, contraceptive injections, sex education and psychological assistance. Women

eligible for STI screening (i.e. at first contact between PASOP and the sex worker or after perceived risk (e.g. condom failure)) were informed on our study and invited to participate, after documenting the informed consent. We intended to include 300 FSW, as preliminary research in the same population on *C. trachomatis* showed that this sample size should result in a representative number of positives (n>20) (4). Post hoc, we calculated the power sample size of our results, using the SAS Power and Sample Size software (SAS Institute Inc., Cary, NC, USA).

Six specimens were sampled consecutively: vaginal Abbott swab (Abbott, Illinois, USA), 89 vaginal Copan FLOQswab (Copan, Brescia, Italy), ThinPrep cervical smear (Hologic Inc., 90 Massachusetts, USA), cervical Abbott swab, cervical Copan FLOQswab and first-void urine 91 collected on Abbott multi-Collect Sample medium. We defined this order based on other 92 scientific literature and after own preliminary research (1-4). Cervical samples were taken 93 94 using a speculum with gel lubricant, which has proven not to influence the quality of the samples. The cervical smear was taken before the cervical swabs, in the light of the correct 95 assessment of cervical cytology. Vaginal swabs were taken prior to the cervical samples, in 96 order to avoid potential dilution by the lubricant. Abbott swabs were sampled before Copan 97 swabs, as the latter showed to have an advantage in detecting more positive samples. Urine 98 samples were accepted at any time of the collection, as long as the FSW respected a one 99 hour time-interval after the last pee. 100

Samples were sent to the Department of Laboratory Medicine of the Ghent University Hospital at room temperature within 24h. Immediately after arrival, the swabs were cultured for *N. gonorrhoeae* on BBL GC-Lect Agar (BD, New Jersey, USA) and read after 48h incubation at 37 °C with 5% CO₂. All cervical smears were sent to the Department of Pathology of the Ghent University Hospital for cervical cytology investigation, using the

106 Bethesda system: high-grade squamous intraepithelial lesion (HSIL), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells, cannot exclude HSIL (ASC-H), atypical 107 cells of undetermined significance (ASC-US) or negative for intraepithelial lesion or 108 malignancy (NILM). Subsequently, all samples were analyzed by NAAT for C. trachomatis, N. 109 110 gonorrhoeae, M. genitalium, T. vaginalis and HPV using the Abbott m2000sp/rt systems and the Abbott RealTime CT/NG kit, the Diagenode S-DiaMGTV qPCR kit (Diagenode, New 111 Jersey, USA) and the Abbott RealTime High Risk HPV kit. As the Abbott HPV kit is intended to 112 detect 14 high risk HPV (hrHPV) genotypes (16/18/31/33/35/39/45/51/52/56/58/ 113 59/66/68), the results will be discussed as negative or positive for hrHPV. All Abbott 114 analyses were performed according to the manufacturers' instructions. For Diagenode, the 115 manufacturer's protocol was slightly modified by adding 5,0 µL Diagenode Optima DU 116 Master Mix 2x DNA; 2,5 µL MGTV double-dye probe and primers; 2,5 µL Double-dye probe 117 118 and primers Universal Inhibition Control (UIC); 2,5 µL UIC and 2,5 µL water to 10 µL DNA extract of each sample. In case of invalid results due to (pre-)analytical errors, the analyses 119 were repeated after 1:2 dilution. In between analyses, all samples were stored at -20 °C. The 120 stability of each infectious agent was verified under different storage conditions (data not 121 published). 122

Given the high positive predictive value of NAAT – the reference technique – confirmatory testing of positive results is not recommended. Therefore, a FSW was considered infected whenever she tested positive on at least one sample type (consensus result). Because this approach cannot generate false positive results, we calculated the sensitivity and not the specificity of each sample type for a given infectious agent. All results were analyzed by SPSS Statistics software v24 using Generalized Estimation Equations and Linear Mixed Models

tests with the Holm–Bonferroni method. The study was approved by the Ethical Committee
of the Ghent University Hospital with Belgian registration number B670201524867.

131

132 **RESULTS**

133 Figure 1 shows the procedure of inclusion and the results. Overall, we executed 1208 bacterial cultures and 8940 NAAT analyses. Internal control failures occurred in 23/8940 134 (0.3%) of NAAT analyses, mainly vaginal Copan swab (n=12) and urine (n=9), necessitating 135 repeat testing which was successful in all cases. Fifteen participants were included twice. In 136 case of repeat positive testing, the possible link was investigated in order to avoid 137 overestimating the prevalence. Overall, 65.6% (196/299) of FSW tested positive for at least 138 one STI on at least one sample type. The individual prevalence rates were 3.3% (10/299) for 139 N. gonorrhoeae; 9.0% (27/299) for C. trachomatis; 7.4% (22/298) for T. vaginalis; 10.8% 140 (32/296) for *M. genitalium* and 55.6% (158/284) for HPV (Figure 1). The mean age [+/-95% 141 confidence interval] of the infected females was 30 years [24-36] for N. gonorrhoeae; 30 142 years [27-33] for *C. trachomatis*, 30 years [27-33] for *T. vaginalis*; 31 years [28-34] for M. 143 genitalium and 32 years [31-33] for HPV. Using the consensus result, we calculated the 144 sensitivities of each sample type for each infectious agent. Figures 1 and 2 show that for N. 145 146 gonorrhoeae culture, cervical Copan swabs showed a significant superiority compared to all other sample types. For all NAAT parameters combined, vaginal swabs detected significantly 147 more cases compared to all other sample types, whilst first-void urine and cervical Abbott 148 149 swabs detected significantly less cases compared to all other sample types. The power of 150 these comparisons is 98.7%.

Taking each infectious agent into account, 19% (56/303) of FSW were co-infected with at
least two STIs. However, when disregarding the HPV results – the most prevalent STI – only
4% (12/303) of FSW were co-infected. Of those, FSW with *N. gonorrhoeae*-infection were
most likely co-infected (6/10), followed by *T. vaginalis* (7/22), *C. trachomatis* (7/27) and *M. genitalium* (5/32).

Remarkably for HPV, both vaginal swabs detected far more cases than all other sample 157 types. In order to clarify this finding, we explored the results of cervical cytology. Overall, 158 cervical cytology revealed 56% (168/302) NILM, 24% (72/302) ASC-US, 12% (35/302) LSIL, 159 7% (21/302) HSIL and 2% (6/302) ASC-H. Matching the HPV results on cervical smear with 160 cervical cytology shows that normal cytology resulted significantly less often in HPV 161 positivity compared to abnormal cytology (Figure 3). In case of normal cytology, vaginal 162 swabs showed a significantly higher positivity rate than all other sample types, whilst in case 163 164 of abnormal cytology, all sample types showed rather comparable rates of HPV positivity (Figure 3). 165

166

167 **DISCUSSION**

We aimed to optimize STI testing by conducting a prospective study in FSW who were tested for *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, *M. genitalium* and HPV on six samples from different anatomical sites. We documented the prevalence of (co-)infections and explored the existence of a consensus sample type.

172

173 The *C. trachomatis* prevalence in this study (9.0%) is slightly higher compared to previous 174 studies in the PASOP population (7.4% and 7.6%), though clearly lower than the worldwide

prevalence rates in FSW (12.5% (range 0.6- 46.2%)) (4-12). The same applies to N. 175 gonorrhoeae and T. vaginalis, for which our prevalence rates (3.3% and 7.4% respectively) 176 are clearly lower than the median prevalence rate reported worldwide amongst FSW (10.7% 177 (range 0- 41.3%) and 13.5% (range 0.1-51.0%) respectively) (4-14). In contrast, the 11.1% 178 prevalence of *M. genitalium* is in line with reports on FSW (13.1% (range 9.2 - 26.3%)) (7, 15, 179 16). As for HPV, the 55.6% prevalence rate is comparable to previous PASOP reports (55.9%) 180 but higher compared to multiple other studies in FSW worldwide (41.1% (range 2.3-100%)) 181 (7, 8, 17, 18). Our prevalence could be overestimated due to the high yield of HPV in vaginal 182 swabs compared to cervical smears, the reference sample type. Indeed, when considering 183 the results on cervical smear as the consensus result, the prevalence falls back to 41.6%. 184 One could presume that a positive STI test following recent sexual activity could be derived 185 from the partner. Although a small study found no effect on HPV detection when vaginal 186 187 intercourse occurred within 48 hours of self-sampling, further studies are needed to evaluate the effect of vaginal intercourse on STI screening and cervical cytology(19). 188

189

STI guidelines recommend the use of various specimens for STI detection: cervical swabs for 190 N. gonorrhoeae culture, vaginal and cervical swabs for N. gonorrhoeae and T. vaginalis, 191 vaginal swabs for C. trachomatis and vaginal swabs or urine for M. genitalium (20-25). Our 192 data show that for *N. gonorrhoeae* culture, cervical Copan swabs were the only acceptable 193 sample type. The inferiority of Abbott swabs for culture was expected, as Abbott transport 194 195 medium contains guanidine thiocyanate, which lyses bacteria and denatures proteins, compromising bacterial growth. Given the inferiority of N. gonorrhoeae culture in 196 comparison to NAAT in detecting N. gonorrhoeae, culture should be reserved for patients 197 198 with persistent infection after treatment to exclude antimicrobial resistance. As for NAAT

testing, testing on vaginal swabs consistently resulted in the highest sensitivities, which offers an opportunity to improve STI screening coverage, as the vast majority of women favors self-sampling over physician-sampling (26). In contrast, first-void urine and cervical Abbott swabs had the lowest sensitivities. It should be noted that urine samples were accepted at any time of the collection with the only restriction being a one hour timeinterval after the last urination. A more stringent approach, using at least 30 mL of firstvoided morning urine and centrifugationcould render more valuable results.

206

Current cervical cancer screening programs are often cytology-based, with HPV testing only 207 as triage of ASC-US positive smears or on follow-up samples. The evidence on the protection 208 against development of cervical (pre-)cancerous lesions collected so far, however, suggests 209 the introduction of HPV testing as a primary screening test. Non-participation is a major 210 211 challenge concerning the effectiveness of screening programs and self-sampling, surely by easy to take vaginal swabs, might be a preferential approach to these non-attendees. 212 Although a cervical smear is considered as the gold standard, vaginal or cervical self-213 sampling methods seem equivalent (27). In fact, HPV may be detected in vaginal sites even 214 before it is detected in the cervix (28). This could explain the high number of positives on 215 vaginal swabs in our study. However, one should take into account that the carcinogenic 216 effects of HPV are not limited to the cervix, but also 43% of vulvar and 70% of vaginal 217 cancers are attributable to HPV (29). Remarkably, our study showed that FSW with HPV 218 219 detected exclusively in the vaginal region were more likely to have normal cervical cytology results. Hence, HPV detected in vaginal swabs could represent freshly infected vaginal cells 220 - whether or not as a prelude of later cervical infection - rather than exfoliation from 221 222 infected cervical cells. Alternatively, HPV detected in vaginal swabs could be derived from

the male sexual partner, whereas the cervical smear reflects the FSW's own HPV condition. 223 In addition, it should be noted that about 90% of HPV infections are asymptomatic and 224 resolve spontaneously within two years (30). As for vaginal swabs, the use of first-void urine 225 for HPV testing assumes contamination with infected exfoliated cervical cells though it may 226 detect urethral or vaginal infections, rather than cervical infections. Indeed, paired cervical 227 and urine samples have showed to detect different types of HPV (31). Our data show that 228 first-void urine is inferior in detecting HPV compared to all other sample types, reflected by 229 both the lower number of positives, which is confirmed by others (31). The lack of 230 standardized methods of urine sampling could be met by using collection devices, though 231 even then cervical smear seems superior for HPV detection (32). 232

233

Some potential limitations need to be mentioned. As close consideration was given to the 234 235 order in which samples were taken, the order was not altered during the study, whereby potential influences on the results cannot be fully excluded. Analyses were conducted 236 following manufacturers' instructions, though not all sample types are cleared for each 237 analysis. This is particularly true for HPV, where clinical cutoffs are applied when 238 interpreting the results. Given the often asymptomatic nature of the selected STIs and 239 excellent test performance of NAAT, we considered a FSW infected whenever she tested 240 positive at least on one sample type, irrespective of signs and symptoms. Finally, as we 241 studied a population of FSW, which are at higher risk for STI than the overall population, 242 caution is to be made when generalizing our results to lower prevalence populations. 243

244

To our knowledge, this is the first study to explore a consensus sample type for STI testing.
The strengths of our study include the simultaneous detection of five STIs and sampling of

different anatomical sites, including swabs of two manufacturers. Apart from HPV, we found 247 relatively low prevalence rates in comparison with other studies conducted in FSW 248 worldwide. This could be explained by geographical differences and/or the influence of a 249 well-organized outreach health program, as FSW are often involved in clandestine practices. 250 251 Vaginal swabs are the preferred sample for STI testing whilst first-void urine should be avoided. In addition to its promising ability to detect STIs, vaginal (self-)sampling should 252 enhance participation in targeted screening programs and in women who refuse or not 253 need a cervical examination, who may be discouraged from testing by the prospect of a 254 speculum examination. The designation of a consensus sample type enables harmonization 255 of STI testing, as it allows for simultaneous analysis of different STIs. This could result in 256 diagnosis and treatment being available more quickly and in a more patient-friendly way, 257 thereby curbing the clinical impact of STIs. The clinical accuracy of HPV on self-collected 258 259 samples needs further investigation. Meanwhile, cervical smears are likely to remain the sample of choice, especially in a cytology based screening program where cervical smears 260 are suitable both for cytology and HPV testing. 261

262

263 **KEY MESSAGES**

- When testing for STIs, vaginal swabs are the sample of choice.

265 - The designation of a non-invasive consensus sample type enables harmonization of

- 266 STI diagnosis, by means of (self-)sampling and simultaneous testing for different STIs.
- Prevalence rates were the highest for HPV, followed by *M. genitalium*, *C. trachomatis*, *T. vaginalis* and *N. gonorrhoeae*.
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- 270

271 **REFERENCES**

Schwebke JR, Hobbs MM, Taylor SN, Sena AC, Catania MG, Weinbaum BS, et al. Molecular
 testing for Trichomonas vaginalis in women: results from a prospective U.S. clinical trial. J Clin
 Microbiol. 2011;49(12):4106-11.

Fang J, Husman C, DeSilva L, Chang R, Peralta L. Evaluation of self-collected vaginal swab,
 first void urine, and endocervical swab specimens for the detection of Chlamydia trachomatis and
 Neisseria gonorrhoeae in adolescent females. J Pediatr Adolesc Gynecol. 2008;21(6):355-60.

278 3. Griffith WF, Stuart GS, Gluck KL, Heartwell SF. Vaginal speculum lubrication and its effects on 279 cervical cytology and microbiology. Contraception. 2005;72(1):60-4.

Coorevits L, Vanscheeuwijck C, Traen A, Bingé L, Ryckaert I, Padalko E. Evaluation of Copan
 FLOQSwab for the molecular detection of Chlamydia trachomatis by Abbott RealTime CT PCR. Acta
 Clin Belg. 2015;70(6):398-402.

283 5. Mak RP, Van Renterghem L, Traen A. Chlamydia trachomatis in female sex workers in
284 Belgium: 1998-2003. Sex Transm Infect. 2005;81(1):89-90.

Verscheijden MM, Woestenberg PJ, Götz HM, van Veen MG, Koedijk FD, van Benthem BH.
 Sexually transmitted infections among female sex workers tested at STI clinics in the Netherlands,
 2006-2013. Emerg Themes Epidemiol. 2015;12:12.

Vielot N, Hudgens MG, Mugo N, Chitwa M, Kimani J, Smith J. The Role of Chlamydia
trachomatis in High-Risk Human Papillomavirus Persistence Among Female Sex Workers in Nairobi,
Kenya. Sex Transm Dis. 2015;42(6):305-11.

291 8. Cwikel JG, Lazer T, Press F, Lazer S. Sexually transmissible infections among female sex
 292 workers: an international review with an emphasis on hard-to-access populations. Sex Health.
 2008;5(1):9-16.

Poon AN, Li Z, Wang N, Hong Y. Review of HIV and other sexually transmitted infections
 among female sex workers in China. AIDS Care. 2011;23 Suppl 1:5-25.

296 10. Cárcamo CP, Campos PE, García PJ, Hughes JP, Garnett GP, Holmes KK, et al. Prevalences of
 297 sexually transmitted infections in young adults and female sex workers in Peru: a national
 298 population-based survey. Lancet Infect Dis. 2012;12(10):765-73.

11. Klingenberg RE, Mannherz S, Brockmeyer NH, Wach J, Winter R, Tiemann C, et al. [Local
health study : Outreach medical services for female sex workers in Bochum]. Hautarzt.
2016;67(12):989-95.

Bremer V, Haar K, Gassowski M, Hamouda O, Nielsen S. STI tests and proportion of positive
 tests in female sex workers attending local public health departments in Germany in 2010/11. BMC
 Public Health. 2016;16(1):1175.

Luo L, Reilly KH, Xu JJ, Wang GX, Ding GW, Wang N, et al. Prevalence and correlates of
 Trichomonas vaginalis infection among female sex workers in a city in Yunnan Province, China. Int J
 STD AIDS. 2016;27(6):469-75.

14. Crucitti T, Jespers V, Mulenga C, Khondowe S, Vandepitte J, Buvé A. Trichomonas vaginalis is
highly prevalent in adolescent girls, pregnant women, and commercial sex workers in Ndola, Zambia.
Sex Transm Dis. 2010;37(4):223-7.

311 15. Gomih-Alakija A, Ting J, Mugo N, Kwatampora J, Getman D, Chitwa M, et al. Clinical
312 characteristics associated with Mycoplasma genitalium among female sex workers in Nairobi, Kenya.
313 J Clin Microbiol. 2014;52(10):3660-6.

16. Pépin J, Labbé AC, Khonde N, Deslandes S, Alary M, Dzokoto A, et al. Mycoplasma
genitalium: an organism commonly associated with cervicitis among west African sex workers. Sex
Transm Infect. 2005;81(1):67-72.

317 17. Soohoo M, Blas M, Byraiah G, Carcamo C, Brown B. Cervical HPV Infection in Female Sex
318 Workers: A Global Perspective. Open AIDS J. 2013;7:58-66.

Mak R, Van Renterghem L, Cuvelier C. Cervical smears and human papillomavirus typing in
 sex workers. Sex Transm Infect. 2004;80(2):118-20.

Harper DM, Longacre MR, Noll WW, Belloni DR, Cole BF. Factors affecting the detection rate
 of human papillomavirus. Ann Fam Med. 2003;1(4):221-7.

323 20. Bignell C, Unemo M, Board ESGE. 2012 European guideline on the diagnosis and treatment
324 of gonorrhoea in adults. Int J STD AIDS. 2013;24(2):85-92.

Prevention CfDCa. Recommendations for the laboratory-based detection of Chlamydia
 trachomatis and Neisseria gonorrhoeae--2014. MMWR Recomm Rep. 2014;63(RR-02):1-19.

327 22. Nwokolo NC, Dragovic B, Patel S, Tong CY, Barker G, Radcliffe K. 2015 UK national guideline
328 for the management of infection with Chlamydia trachomatis. Int J STD AIDS. 2016;27(4):251-67.

Lanjouw E, Ouburg S, de Vries HJ, Stary A, Radcliffe K, Unemo M. 2015 European guideline
on the management of Chlamydia trachomatis infections. Int J STD AIDS. 2016;27(5):333-48.

331 24. Sherrard J, Ison C, Moody J, Wainwright E, Wilson J, Sullivan A. United Kingdom National
332 Guideline on the Management of Trichomonas vaginalis 2014. Int J STD AIDS. 2014;25(8):541-9.

S. JJ, M. C, M. G. 2016 European guideline on Mycoplasma genitalium infections. Norway:
Prof Harald Moi, Osla University Hospital and Faculty of Medecing, University of Oslo, Norway; 2016.

Schachter J, Chernesky MA, Willis DE, Fine PM, Martin DH, Fuller D, et al. 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):725-8.

Petignat P, Faltin DL, Bruchim I, Tramèr MR, Franco EL, Coutlée F. Are self-collected samples
 comparable to physician-collected cervical specimens for human papillomavirus DNA testing? A
 systematic review and meta-analysis. Gynecol Oncol. 2007;105(2):530-5.

Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus
infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol.
2003;157(3):218-26.

Taylor S, Bunge E, Bakker M, Castellsagué X. The incidence, clearance and persistence of
non-cervical human papillomavirus infections: a systematic review of the literature. BMC Infect Dis.
2016;16:293.

348 30. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal 349 papillomavirus infection in young women. N Engl J Med. 1998;338(7):423-8.

350 31. Sehgal A, Gupta S, Parashari A, Sodhani P, Singh V. Urine HPV-DNA detection for cervical 351 cancer screening: prospects and prejudices. J Obstet Gynaecol. 2009;29(7):583-9.

32. Vorsters A, Van Keer S, Biesmans S, Hens A, De Coster I, Goossens H, et al. Long-Term
Follow-up of HPV Infection Using Urine and Cervical Quantitative HPV DNA Testing. Int J Mol Sci.
2016;17(5).

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357 FIGURE LEGENDS

- 358 Figure 1 – Flowchart of inclusion of FSW and number of positive results for each parameter on the different sample types. (NGc = Neisseria gonorrhoeae culture; NG= Neisseria 359 gonorrhoeae; CT= Chlamydia trachomatis; TV = Trichomonas vaginalis; MG = Mycoplasma 360 *genitalium*; HPV = human papilloma virus; FVU= first void urine; VA = vaginal Abbott swab; 361 VC = vaginal Copan swab; CA = cervical Abbott swab; CC cervical Copan swab; CS = cervical 362 smear; ^a significantly higher than CA (p<0.05); ^b significantly higher than FVU (p<0.05); ^c 363 significantly higher than CC (p<0.05); ^d significantly higher than CS (p<0.05); ^e significantly 364 higher than VA (p<0.05); ^f significantly higher than VC (p<0.05); grey background indicates 365 sample type with highest sensitivity) 366 367 Figure 2 – Percentage of infections detected (■) or missed (■) for each parameter on the 368 different sample types. (FVU= first void urine; VA = vaginal Abbott swab; VC = vaginal Copan 369 swab; CA = cervical Abbott swab; CC = cervical Copan swab; CS = cervical smear) 370 371 372 Figure 3 – Analysis of high-risk HPV results in case of normal versus abnormal cytology. 373 ^a significantly higher than FVU (p<0.05); ^b significantly higher than CA (p<0.05); ^c significantly 374
- higher than CC (p<0.05); ^d significantly higher than CS (p<0.05)
- 376
- 377

378 TRANSPARENCY DECLARATION

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- 390



Gc positive n = 4	NG positive n = 10	CT posit n = 27	tive TV po 7 n =	ositive MG p = 23 n		sitive 35	HPV positive n = 166	At leas	least one positive n = 196	
	FVU 50% [l: n = 5 22-78%]	FVU: n = 19 70% [51-84%]	FVU: r 74% [5:	n = 17 1-87%]	→ FVU: n = 63% [46-7	22 8%] → FVU: 61% [n = 101 51-67%]	FVU: n = 1 66% [59-72	
→ VA: n 0% [0	= 0 -0%] VA:	: n = 8 46-95%]	VA: n = 20 74% [55-87%]	VA: n 83% [6:	VA: n = 19 83% [61-93%]		18 1%] → VA: n = 87% [= 145 ^{a,b,c,d} 80-90%]	→ VA: n = 174ª 88% [83-92	
VC: n 25% [3	= 1 VC: -76%] VC:	n = 6 30-84%]	VC: n = 22 ^a 81% [62-92%]	VC: n 78% [50	= 18 5-90%]	VC: n = 3 86% [70-9	60 5%] → VC: n = 91% [= 151 ^{a,b,c,d} 84-93%]	VC: n = 178ª 90% [85-94	
CA: n 0% [0	= 0 CA: -0%] CA:	: n = 4 16-70%]	CA: n = 15 56% [37-73%]	CA: n 74% [5:	= 17 1-87%]	CA: n = 2 74% [57-8	:6 8%] → CA: 1 70% [n = 116 61-75%]	CA: n = 14 71% [65-77	
CC: n =	e 4ª,e,f 0-100%] CC: 40% [: n = 4 16-70%]	CC: n = 18 67% [47-82%]	CC: n 78% [5	= 18 6-90%]	→ CC: n = 2 71% [55-8	!5 6%] → CC: n 78% [= 129 ^{a,b} 69-82%]	CC: n = 156	
-	CS:	: n = 7 38-90%]	CS: n = 21	CS: n	= 18	CS: n = 2	1%] CS: n	n = 125 ^b 67-80%]	CS: n = 155	

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