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Detection of HPV mRNA in Self-collected Vaginal Samples Among Women at 69-70 Years of Age

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Abstract. *Background/Aim: Cervical cancer is associated with poorer diagnosis among the elderly and pap-smear screening has a lower sensitivity. Self-sampling for detection of high-risk human papillomavirus (hr-HPV) may be an alternative screening method. The aim of this study was to analyze the response rate to vaginal HPV self-sampling and the HPV mRNA prevalence among women 69-70 years. Materials and Methods: An HPV self-sampling kit was sent to 1,000 women 69-70 years whom had not taken a cervical smear in ≥5 years. The samples were analyzed by the Aptima HPV mRNA assay. HPV-positive women were recalled for a follow-up examination. Results: The self-sample response rate was 43.3%. The HPV mRNA prevalence was 6.2%. All HPV-positive women attended follow-up. Conclusion: HPV self-sampling was accepted among older women. Although the HPV mRNA prevalence was 6.2%, no high-grade cytological abnormalities were found. Larger studies are needed to elucidate hr-HPV self-sampling as a tool to identify older women at risk of cervical cancer.*

In 2017 one out of five people were 65 years or older in Sweden and the number of people 80 years and older are expected to increase by 50% in less than 30 years (1). Cervical cancer is one of the most common cancers among women globally (2). Some studies show a bimodal distribution of cervical cancer incidence with a first peak among younger women around the age of 40 and a second peak among older women around the age of 65-79 (3, 4). In a Danish study, it was observed that the peak incidence of cervical cancer will shift to 75-79 years of age when correcting for hysterectomy (4). Considerations must be taken because cervical cancer is not only a disease of young women, but also an important disease in the elderly. Several previous studies have shown that older women to a greater extent are diagnosed with an advanced-stage disease (3, 5-9) and the prognosis is poor (7, 8). A pap smear has a lower sensitivity among postmenopausal women (10) as the transformation zone moves upwards into the cervical canal (11, 12) in this age group, contributing to a higher risk of clinical sampling errors which may miss women with cervical dysplasia or invasive cancer.

There is no consensus at what age to stop screening for cervical cancer. According to European guidelines, screening with cytology or human papillomavirus (HPV) could be stopped around 60-65 years, given the woman has had a recent negative test (13). In Sweden, the organized screening program for cervical cancer comprises women 23-64 years old, but the women must be recalled at the age of 70 years if no test is registered after the age of 64 years (11). A paradigm shift for cervical cancer screening is currently under way in several countries. Persistent infection with high-risk HPV (hr-HPV) is the main reason for 99.7% of cervical dysplasia and invasive cervical cancer (14). Screening for hr-HPV types has been shown to be more effective in preventing invasive cervical cancer compared to cytology for women ≥30 years, hence this method is now recommended as primary screening method (15, 16). In the county Region Skåne, Sweden, primary HPV mRNA screening is used for women ≥30 years since January 2017 (17). Another advantage of the HPV testing is the possibility to perform it as a self-sampling procedure which can be one method to reach women not participating in routine screening (18). There are several hr-HPV tests available, HPV mRNA tests have been shown to have similar sensitivity as the HPV DNA tests, but with superior specificity in detecting precancerous cervical lesions (19-21).

Given the fact that life expectancy is increasing, it is of importance to increase knowledge of prevalence of hr-HPV infections in postmenopausal women and their need of further screening in order to prevent the development of

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cervical dysplasia or cancer. Since a single pap-smear has a lower sensitivity among older women and in order to increase the availability of screening, HPV self-sampling could be an alternative or additional tool to the regular screening procedure. The aim of this study was to analyze the response rate of a free-of-charge offered vaginal HPV self-sample sent to home as well as the HPV mRNA prevalence among women 69-70 years of age which is the upper screening limit in Sweden.

Materials and Methods

One thousand women in the region of Lund (community of Lund, Eslöv and Höör) 69-70 years of age whom had not taken a cervical smear in five years or more were identified through the Southern regional cervical screening registry and invited to participate in the study. The screening registry contains information on all taken smears, organized or spontaneously taken, in the region. A kit for HPV self-sampling was sent to the women through regular post on April 25, 2017. The self-sampling parcel contained; 1) one information chart with invitation and instructions of how to take the self-sample, 2) one Aptima Multitest Swab and a tube prefilled with 2.9 ml Aptima Multitest Swab Transport Media (STM) (Hologic Inc, Marlborough, MA, USA), 3) one cylindrical container for transportation of the self-sample, 4) two labels with the social security number to mark the test (one at the self-sampling test and one at the cylindrical container) and 5) one prepaid padded return envelope addressed to the Laboratory Medicine, Region Skåne, Lund. The self-sample was collected by placing the cotton swab 3-4 cm up into the vagina and rotate it 360 degrees 2-3 times, thereafter the cotton swab was put in the tube containing transport media. The women were asked to carefully check that the social security number in the kit was correct to reduce the risk of a wrong woman taking the test. The labels with the social security number were used as identification during the analytical process. The information chart instructed the women to send in the self-sample one week after receiving the kit. No reminder was sent out if the kit was not sent in. The self-sampling test was voluntary and free of charge for the women, no financial compensation was paid. The Laboratory Medicine, Region Skåne, Lund received the self-samples and conducted the HPV analyses. All the self-samples were analyzed by Aptima HPV mRNA assay (Hologic Inc.) on a Panther instrument, according to the manufacturer's instructions. The assay detects HPV mRNA from 14 hr-HPV types [16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68].

Women with a negative HPV test result received an automatic generated letter from the Laboratory Medicine containing the information that no hr-HPV types were found and that no further testing was needed. In case of a positive HPV test, the information was sent to the Women's outpatient clinic in Lund which forwarded the test result by a letter to the affected woman and informed about the necessity to perform a clinical follow-up examination. The follow-up examination was performed according to the current screening guidelines for cervical cancer applied in the region of Skåne (including the region of Lund). A liquid based cytology (LBC) sample was taken by a clinician from the cervix for HPV mRNA testing and cytological analysis. In case of abnormal test results at the follow-up examination, the women were further treated according to current guidelines.

The study was approved by the Regional Ethical Review Board, Lund (DNR 2013/390).

Statistical analyses. Statistical comparisons were based on the binomial distribution and the exact confidence intervals (CI) are given. Microsoft® Excel, Version 15.30 was used on a Mac computer for the statistical analyses.

Results

The last self-sample returned was registered 10 weeks and 6 days from sent out date. The response rate of the self-sample was 43.3% [(433/1,000), 95%CI=40.2-46.4]. The prevalence of HPV mRNA was 6.2% ((27/433), 95%CI=4.1-8.9). Initially 55 self-samples (12.7%, 95%CI=9.7-16.2) were found invalid by the Aptima HPV assay. After limited reanalysis of eight invalid samples, only two samples were found valid. Therefore, the remaining 53 invalid samples were diluted ¼ (1 ml sample were transferred to 2.9 ml Aptima Transfer Solution), rendering 52 samples valid. The residual invalid sample was valid after being diluted to 1/8 (1 ml from the ¼ dilution sample was transferred to 2.9 ml Aptima Transfer Solution). All the 55 initially invalid samples were HPV mRNA negative.

All the 27 HPV mRNA positive women attended the follow-up examination. Six out of 27 (22.2%, 95%CI=8.6-42.3) women were HPV mRNA positive in the cervical HPV sample. Two out of 27 (7.4%, 95%CI=0.9-24.3) women had atypical cells of undetermined significance (ASCUS) at cytology and 25 women had benign cytology. Out of women with ASCUS, one had a positive cervical HPV mRNA test and was invited for colposcopy and cervical biopsy three months later, which showed no dysplasia. The other woman with ASCUS had a negative cervical HPV mRNA test and was invited in twelve months for a new LBC-test and a new cervical HPV test, which showed no cytological dysplasia and negative HPV mRNA. The study design and occurrence of HPV mRNA and dysplasia is shown in Figure 1.

Discussion

This study found an HPV mRNA prevalence of 6.2% among women 69-70 years old by self-sampling within a county of southern Sweden. Without reminding letter, 43% submitted their self-sample. All HPV positive women attended follow-up examination. No high-grade cytological abnormalities were found.

The HPV mRNA prevalence of 6.2% among our self-sampled women 69-70 years old is in good concordance with previous studies. Two American studies and one study from the east of Sweden have found hr-HPV DNA prevalence for postmenopausal women to be between 6.0-6.2% (10, 22, 23). Other studies have found a slightly lower hr-HPV DNA prevalence for older women ranging from 2.9-5.6% (12, 21,

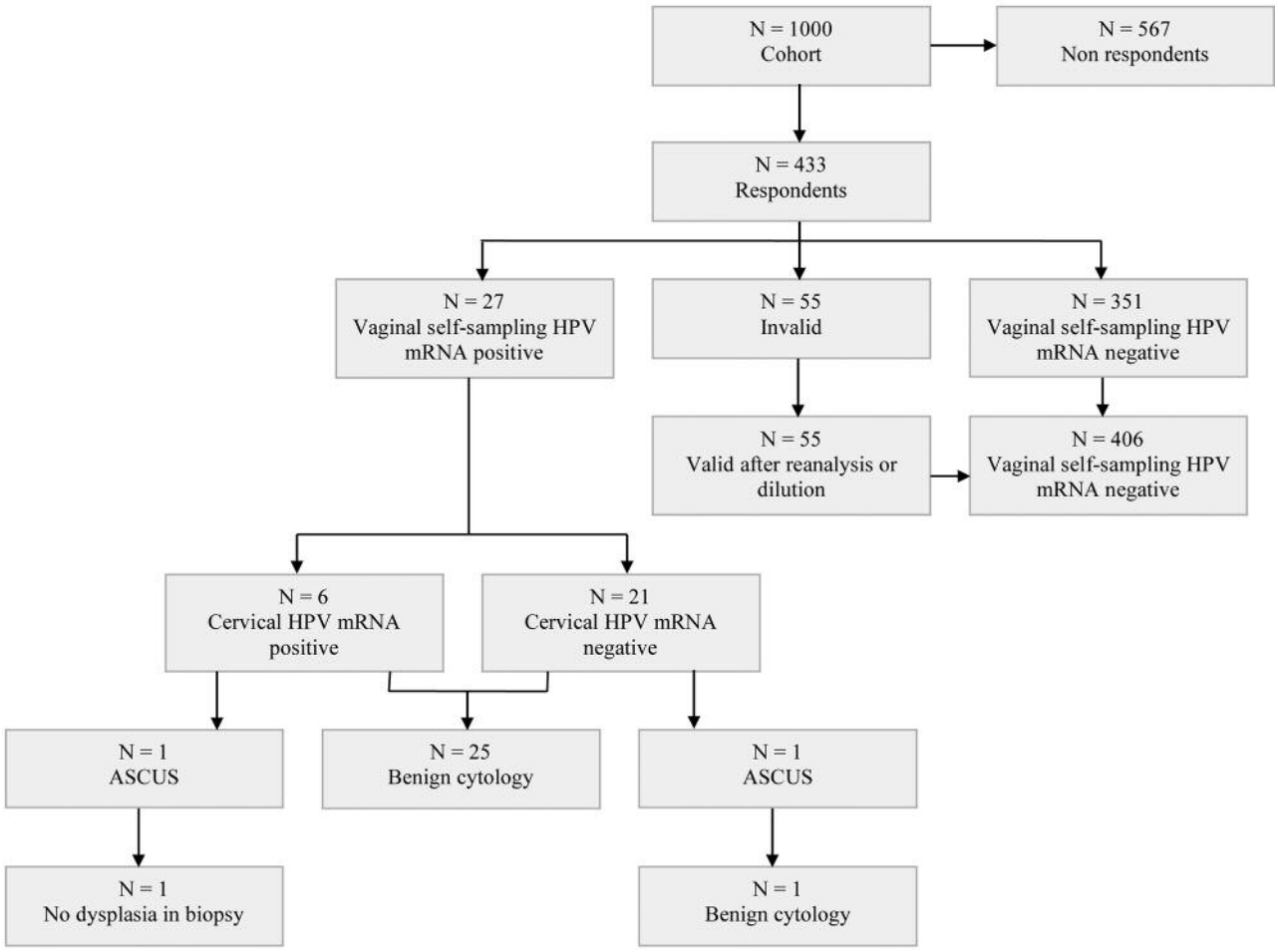

Figure 1. *Flow-chart showing study design and occurrence of HPV mRNA and dysplasia. HPV: Human papillomavirus; ASCUS: atypical cells of undetermined significance.*

24-26). The screening history of the participating women varied in the studies mentioned above. However, Lindau *et al*. showed similar hr-HPV prevalence in postmenopausal women regardless of time since last screening test (22). Several previous studies stated that hr-HPV type prevalence is relatively low among older women (24-28). The overall hr-HPV prevalence in western regions in the world is estimated between 10.0-11.3% (27, 29). Even though our study found a lower hr-HPV prevalence among older women, a self-sampled HPV mRNA prevalence of 6.2% may indicate that this age group is at risk of developing a persistent hr-HPV infection which may lead to the development of precancerous lesions or invasive cancer of the cervix. However, considerations must be taken because no high-grade cytological abnormalities were found in this study. Of interest though, Hermansson *et al*. recently reported histologically verified cervical intraepithelial neoplasia (CIN) 1 and 2 among 86.4% of older women (60-89 years) with persistent hr-HPV infection and normal cytology, suggesting that cytology has very low sensitivity among this sub-group of women (12).

All HPV positive women in this study attended the follow-up examination. A good attendance rate for follow-up of HPV positive women is essential if hr-HPV self-sampling is to be used. Previous studies in Sweden have found an attendance rate between 70-100% for follow-up examination (30-32). At the follow-up examination in our study only six out of 27 women were HPV mRNA positive in the cervical sample. Interestingly the hr-HPV positivity has been demonstrated to be 38% higher in vaginal self-samples compared to cervical samples among a population of women aged ≥50 years, probably because of menopausal changes in the cervix (33). A difference in accuracy between a vaginal- and a cervical HPV test has also been discussed.

A large meta-analysis by Arbyn *et al*. concluded that a cervical hr-HPV test may be superior to a vaginal hr-HPV self-sample (18). However, a recent study validated the HPV mRNA test used in this study to be equivalent in sensitivity to that of cytology in detection of dysplasia (34). Even though only two cases of ASCUS were detected in our series, it is important to mention that the vaginal HPV self-sample was positive in all of them. Furthermore, two other studies stated a high agreement for hr-HPV detection between vaginal HPV self-sampling and clinician-taken cervical HPV samples, with at least one study including older women (35, 36). The quite large decrease in HPV positivity observed in our study might also be explained by a clearance rate of hr-HPV infection in older women. Previous studies have found a high hr-HPV clearance rate for middle-age and older women, varying between 37.2 - 43% during a surveillance period of maximal 6 months (12, 37). However, it has been shown that one in ten women who appear to have cleared an HPV-16 (hr-HPV) infection may be latently infected (38).

Out of 1,000 women in this study, 43.3% chose to send in their HPV self-sample test. In a study by Lindau *et al*. the participation rate for HPV self-sampling in women 57-80 years was 67.5%. A possible reason for their higher participation rate could be the fact that the women were introduced to the self-sampling procedure by field staff with the possibility to ask questions (39). However, these participation rates for older women is higher than in many previous studies looking at attendance rate of self-sampling in all age categories for women not regularly attending cervical screening. Earlier, we have found a participation rate of 14.7% in women whom had not taken a pap smear in nine years or more (30). Broberg *et al*. found a participation rate of 16.0% after a second reminder, however these women had to order the self-kit themselves and had to pay a low fee for the test (32). A study by Rossi *et al*. found that a directly sent out self-kit contributed to a higher participation rate (40). However, this is not in concordance with a Swedish study from Uppsala showing a rather high participation rate of 39.1% for women having to order the self-kit themselves (26). These varying results are interesting, especially since our present and earlier studies are conducted in the same area. A difference in time since the last pap smear may be a reason to the higher participation rate in our present study since Broberg *et al*. concluded that women having had a pap smear within the last 10 years were more likely to attend to self-sampling (32). Other factors in favor of participation to self-sampling are shown to be younger age (<74 years), higher education, recent gynecological examination, use of menopausal hormone and sexual activity in the past year (39).

One limitation of our study is that the invitations were sent out only once without any reminder. Another limitation is that no lifetime information regarding the women's earlier screening history, earlier status of cervical dysplasia, health status, sexual history, usage of postmenopausal hormone regimes or education level was collected. It was neither known if the women had undergone a total hysterectomy, in which case it is known that no cervical screening is needed (11). Lindau *et al*. found that the most common reasons for not taking a self-sampling test were a physical or health problem (39). These factors could also have contributed to non-participation in our study. A strength of the study was the high participation rate and that all self-samples were analyzed. Although, a substantial proportion of the self-samples (12.7%) were initially invalid by the Aptima HPV mRNA assay, but became approved after dilution. The reason for invalid self-samples is unknown to us but recently we decreased the proportion of invalid self-samples (<2%) by the use of a pre-heating step at 90˚C for 1h in a heating chamber (manuscript in preparation).

Our results indicate that HPV self-sampling appears to be accepted to a large extent among older women earlier not in the cervical screening program. An HPV mRNA prevalence of 6.2% among women 69-70 years old was found. Although this indicates that women in age to exit the cervical screening program are at risk of developing a persistent hr-HPV-infection which may lead to the development of cervical dysplasia or invasive cancer, no high-grade cytological abnormalities were found. Larger studies are needed to further elucidate the potential of hr-HPV self-sampling as a tool to identify older women at risk of cervical cancer.

Conflicts of Interest

Hologic Inc. provided the sample kits and did not charge for the HPV analyses performed at the Laboratory in Lund, Sweden. Hologic Inc. did not have any influence on the study design, statistical analyses or manuscript writing. Ola Forslund has received a speech honorarium from Hologic and his laboratory department (Laboratory Medicine, Region Skåne, Lund) has ongoing contracts with Hologic.

None of the other Authors have any conflicts of interest to declare.

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References

1 Statistics Sweden, The future population of Sweden 2018–2070. Demographic reports 2018:1.

2 Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin *61(2)*: 69-90, 2011.

3 Brun JL, Stoven-Camou D, Trouette R, Lopez M, Chene G and Hocke C: Survival and prognosis of women with invasive cervical cancer according to age. Gynecol Oncol *91(2)*: 395-401, 2003.

4 Hammer A, Kahlert J, Rositch A, Pedersen L, Gravitt P, Blaakaer J and Soegaard M: The temporal and age-dependent patterns of hysterectomy-corrected cervical cancer incidence rates in denmark: A population-based cohort study. Acta Obstet Gynecol Scand *96(2)*: 150-157, 2017.

5 Fedewa SA, Cokkinides V, Virgo KS, Bandi P, Saslow D and Ward EM: Association of insurance status and age with cervical cancer stage at diagnosis: National cancer database, 2000-2007. Am J Public Health *102(9)*: 1782-1790, 2012.

6 Eggemann H, Ignatov T, Geyken CH, Seitz S and Ignatov A: Management of elderly women with cervical cancer. J Cancer Res Clin Oncol *144(5)*: 961-967, 2018.

7 Wright JD, Gibb RK, Geevarghese S, Powell MA, Herzog TJ, Mutch DG, Grigsby PW, Gao F, Trinkaus KM and Rader JS: Cervical carcinoma in the elderly: An analysis of patterns of care and outcome. Cancer *103(1)*: 85-91, 2005.

8 Darlin L, Borgfeldt C, Widen E and Kannisto P: Elderly women above screening age diagnosed with cervical cancer have a worse prognosis. Anticancer Res *34(9)*: 5147-5151, 2014.

9 Fox KV, Shah CA, Swisher EM, Garcia RL, Mandel LS, Gray HJ, Swensen RE and Goff BA: An evaluation of cervical cancer in women age sixty and over. Gynecol Oncol *109(1)*: 53-58, 2008.

10 Gyllensten U, Gustavsson I, Lindell M and Wilander E: Primary high-risk hpv screening for cervical cancer in post-menopausal women. Gynecol Oncol 125(2): 343-345, 2012.

11 Regionala Cancercentrum I Samverkan. Cervixcancerprevention. Nationellt vårdprogram och Konsekvenser av införande av Socialstyrelsens rekommendationer gällande screening juni 2015. 2017-01-05. https://www.cancercentrum.se/samverkan/vara-uppdrag/prevention-och-tidig-upptackt/gynekologisk-cellprovskontroll/vardprogram/

12 Hermansson RS, Olovsson M, Hoxell E and Lindstrom AK: Hpv prevalence and hpv-related dysplasia in elderly women. PLoS One *13(1)*: e0189300, 2018.

13 von Karsa L, Arbyn M, De Vuyst H, Dillner J, Dillner L, Franceschi S, Patnick J, Ronco G, Segnan N, Suonio E, Törnberg S and Anttila A: European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on hpv screening and vaccination. Papillomavirus Res *1*: 22-31, 2015.

14 Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ and Munoz N: Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol *189(1)*: 12-19, 1999.

15 Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, Koliopoulos G, Naucler P, Sankaranarayanan R and Peto J: Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine *30*: F88-99, 2012.

16 Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJF, Arbyn M, Kitchener H, Segnan N, Gilham C, Giorgi-Rossi P, Berkhof J, Peto J and Meijer CJLM: Efficacy of hpv-based screening for prevention of invasive cervical cancer: Follow-up of four european randomised controlled trials. Lancet *383(9916)*: 524-532, 2014.

17 Pedersen K, Fogelberg S, Thamsborg LH, Clements M, Nygard M, Kristiansen IS, Lynge E, Sparen P, Kim JJ and Burger EA: An overview of cervical cancer epidemiology and prevention in scandinavia. Acta Obstet Gynecol Scand *97(7)*: 795-807, 2018.

18 Arbyn M, Verdoodt F, Snijders PJF, Verhoef VMJ, Suonio E, Dillner L, Minozzi S, Bellisario C, Banzi R, Zhao F-H, Hillemanns P and Anttila A: Accuracy of human papillomavirus testing on self-collected *versus* clinician-collected samples: A meta-analysis. Lancet Oncol *15(2)*: 172-183, 2014.

19 Cuzick J, Cadman L, Mesher D, Austin J, Ashdown-Barr L, Ho L, Terry G, Liddle S, Wright C, Lyons D and Szarewski A: Comparing the performance of six human papillomavirus tests in a screening population. Br J Cancer *108(4)*: 908-913, 2013.

20 Arbyn M, Snijders PJ, Meijer CJ, Berkhof J, Cuschieri K, Kocjan BJ and Poljak M: Which high-risk hpv assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect *21(9)*: 817-826, 2015.

21 Asciutto KC, Forslund O and Borgfeldt C: Prevalence of high-risk hpv in postmenopausal women with benign cervical cytology – a population-based cohort study. Anticancer Res *38(7)*: 4221-4228, 2018.

22 Lindau ST, Drum ML, Gaumer E, Surawska H and Jordan JA: Prevalence of high-risk human papillomavirus among older women. Obstet Gynecol *112(5)*: 979-989, 2008.

23 Smith EM, Ritchie JM, Levy BT, Zhang W, Wang D, Haugen TH and Turek LP: Prevalence and persistence of human papillomavirus in postmenopausal age women. Cancer Detect Prev *27(6)*: 472-480, 2003.

24 Jacobs MV, Walboomers JM, Snijders PJ, Voorhorst FJ, Verheijen RH, Fransen-Daalmeijer N and Meijer CJ: Distribution of 37 mucosotropic hpv types in women with cytologically normal cervical smears: The age-related patterns for high-risk and low-risk types. Int J Cancer *87(2)*: 221-227, 2000.

25 Wikstrom I, Stenvall H and Wilander E: Low prevalence of high-risk hpv in older women not attending organized cytological screening: A pilot study. Acta Derm Venereol *87(6)*: 554-555, 2007.

26 Sanner K, Wikstrom I, Strand A, Lindell M and Wilander E: Self-sampling of the vaginal fluid at home combined with high-risk hpv testing. Br J Cancer *101(5)*: 871-874, 2009.

27 de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N and Bosch FX: Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: A meta-analysis. Lancet Infect Dis *7(7)*: 453-459, 2007.

28 Smith JS, Melendy A, Rana RK and Pimenta JM: Age-specific prevalence of infection with human papillomavirus in females: A global review. J Adolesc Health *43(4)*: S5-25, S25.e21-41, 2008.

29 Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX and de Sanjose S: Cervical human papillomavirus prevalence in 5 continents: Meta-analysis of 1 million women with normal cytological findings. J Infect Dis *202(12)*: 1789-1799, 2010.

30 Darlin L, Borgfeldt C, Forslund O, Henic E, Hortlund M, Dillner J and Kannisto P: Comparison of use of vaginal hpv self-sampling and offering flexible appointments as strategies to reach long-term non-attending women in organized cervical screening. J Clin Virol *58(1)*: 155-160, 2013.

31 Gyllensten U, Sanner K, Gustavsson I, Lindell M, Wikstrom I and Wilander E: Short-time repeat high-risk hpv testing by self-sampling for screening of cervical cancer. Br J Cancer *105(5)*: 694-697, 2011.

32 Broberg G, Gyrd-Hansen D, Miao Jonasson J, Ryd ML, Holtenman M, Milsom I and Strander B: Increasing participation in cervical cancer screening: Offering a hpv self-test to long-term non-attendees as part of racomip, a swedish randomized controlled trial. Int J Cancer *134(9)*: 2223-2230, 2014.

33 Stanczuk G, Baxter G, Currie H, Lawrence J, Cuschieri K, Wilson A and Arbyn M: Clinical validation of hrhpv testing on vaginal and urine self-samples in primary cervical screening (cross-sectional results from the papillomavirus dumfries and galloway-pavdag study). BMJ Open *6(4)*: e010660, 2016.

34 Asciutto KC, Ernstson A, Forslund O and Borgfeldt C: Self-sampling with hpv mrna analyses from vagina and urine compared with cervical samples. J Clin Virol *101*: 69-73, 2018.

35 Coorevits L, Traen A, Binge L, Van Dorpe J, Praet M, Boelens J and Padalko E: Are vaginal swabs comparable to cervical smears for human papillomavirus DNA testing? J Gynecol Oncol *29(1)*: e8, 2018.

36 Gravitt PE, Lacey JV, Jr., Brinton LA, Barnes WA, Kornegay JR, Greenberg MD, Greene SM, Hadjimichael OC, McGowan L, Mortel R, Schwartz PE, Zaino R and Hildesheim A: Evaluation of self-collected cervicovaginal cell samples for human papillomavirus testing by polymerase chain reaction. Cancer Epidemiol Biomarkers Prev *10(2)*: 95-100, 2001.

37 Bulkmans NW, Berkhof J, Bulk S, Bleeker MC, van Kemenade FJ, Rozendaal L, Snijders PJ, Meijer CJ and Group PS: High-risk hpv type-specific clearance rates in cervical screening. Br J Cancer *96(9)*: 1419-1424, 2007.

38 Korostil IA and Regan DG: The potential impact of hpv-16 reactivation on prevalence in older australians. BMC Infect Dis *14*: 312, 2014.

39 Lindau ST, Hoffmann JN, Lundeen K, Jaszczak A, McClintock MK and Jordan JA: Vaginal self-swab specimen collection in a home-based survey of older women: Methods and applications. J Gerontol B Psychol Sci Soc Sci *64*: i106-118, 2009.

40 Giorgi Rossi P, Marsili LM, Camilloni L, Iossa A, Lattanzi A, Sani C, Di Pierro C, Grazzini G, Angeloni C, Capparucci P, Pellegrini A, Schiboni ML, Sperati A, Confortini M, Bellanova C, D'Addetta A, Mania E, Visioli CB, Sereno E, Carozzi F and Self-Sampling Study Working G: The effect of self-sampled hpv testing on participation to cervical cancer screening in italy: A randomised controlled trial (isrctn96071600). Br J Cancer *104(2)*: 248-254, 2011.

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