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## Comparative Performance of Anyplex II HPV28 and Cobas 4800 Human Papillomavirus (HPV) Assays for High-Risk HPV Detection in Self-collected Anal Samples

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### ► To cite this version:

Gauthier Delvallez, Sokleaph Cheng, Stéphane Marot, Gervillien Arnold Malonga, Théophile Cocherie, et al.. Comparative Performance of Anyplex II HPV28 and Cobas 4800 Human Papillomavirus (HPV) Assays for High-Risk HPV Detection in Self-collected Anal Samples. *Open Forum Infectious Diseases*, 2023, 10 (11), pp.ofad540. 10.1093/ofid/ofad540 . hal-04400991

**HAL Id: hal-04400991**

**<https://hal.science/hal-04400991>**

Submitted on 17 Jan 2024

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1 **TITLE:** Comparative performance of Anyplex II HPV28 and Cobas 4800 HPV assays for high-  
2 risk HPV detection in self-collected anal samples

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4 **RUNNING TITLE:** Comparative HPV assays in self-collected anal samples

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26 **ABSTRACT**

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28           We compared 2 HPV assays to detect the 14 hrHPV genotypes in self-collected anal  
29 samples. We found a good agreement and similar performance to detect HPV16, HPV18 and  
30 the 12 others hrHPV genotypes. The global performance to detect the 14 hrHPV genotypes  
31 was not significantly different between the 2 assays.

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33 **KEYWORDS**

34 Anal lesions; self-sampling; HPV-testing; Anyplex II HPV28; Cobas 4800; HPV16; MSM;  
35 TWG; Cambodia

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## 51 **BACKGROUND**

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53         Around 30 000 new cases of anal squamous cell carcinoma (ASCC) are diagnosed  
54 each year worldwide [1]. Key-population, such as men having sex with men (MSM) and  
55 people living with HIV (PLWHIV), have an increased risk of developing ASCC. For instance,  
56 HIV-infected MSM has the highest incidence rate of ASCC with 85 new cases per 100 000  
57 person-year [2]. Human Papillomavirus (HPV) is involved in roughly 90% of ASCC, of  
58 whom 90% are HPV16 positive. ASCC is preceded by precancerous lesions, i.e. anal  
59 intraepithelial neoplasia 1, 2 and 3 (AIN1, AIN2 and AIN3), AIN1 being identify as low  
60 grade anal lesions (LGAIN), AIN2 and AIN3 as high grade anal lesions (HGAIN). However  
61 for now, high-risk HPV testing (hrHPV) is not recommended for the screening of anal  
62 precancerous and cancer lesions, while it has been clinically validated with multiple hrHPV  
63 tests in cervical cancer screening [3,4]. Although anal HPV16 genotyping is not yet a widely  
64 accepted molecular marker in anal cancer screening, it is by far the most carcinogenetic type  
65 in the anus [5]. Indeed, (i) HPV16 prevalence increases across the anal lesion from normal to  
66 precancerous and anal cancer [6] and (ii) in a recent prospective study, HGAIN HPV16-  
67 positive are less likely to clear compared to those with other hrHPV genotypes [7].

68         In this study, we aim to compare the agreement and performance of the Cobas 4800  
69 HPV-test (Roche®) and the Anyplex<sup>TM</sup>II HPV28 Detection (Seegene Inc) to detect hrHPV in  
70 self-sampling anal swabs from asymptomatic MSM and transgender women (TGW) in  
71 Phnom Penh, Cambodia; especially for HPV16 detection which was the most prevalent HPV  
72 genotype found in this population [8].

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## 76 **MATERIALS AND METHODS**

### 77 **Study population**

78 A cross-sectional retrospective study was conducted between October 1<sup>st</sup>, 2021 and  
79 November 30<sup>th</sup>, 2021 in different clinics in Phnom Penh, Cambodia and under the scope of  
80 activities of Men's Health Cambodia, a non-governmental and non-profit organization  
81 working on men, including men having sex with men (MSM) and transgender women (TGW)  
82 issues in Cambodia. During consultation, a questionnaire was used to collect patient data and  
83 the anal collection (FLOQSwabs, Copan) was self-performed using anal swabs discharged in  
84 Viral Transport Media (VTM). They were then stored at -80°C at the Institut Pasteur du  
85 Cambodge (IPC) until further analysis. A comprehensive explanation of eligibility criteria and  
86 procedures has been published before [8].

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### 88 **HPV-testing and methods' comparison**

89 First analysis was performed at IPC on 400 µl of sample with the Cobas 4800 HPV-test  
90 (Roche®) which allows the detection of 14 hrHPV types by PCR and nucleic acid  
91 hybridization. This test specifically identifies HPV16, HPV18 and 12 others hrHPV in the  
92 same channel including HPV31-33-35-39-45-51-52-56-58-59-66-68. Then, 1ml of frozen  
93 VTM was sent to the Virology Department of the Pitié-Salpêtrière hospital, Paris, France.  
94 DNA-extraction (from 200 µl of sample to 100 µl) was performed with the E-mag device  
95 (Biomérieux) and both samples and extracted DNA were stored at -80°C. Then HPV-testing  
96 was performed with the Anyplex<sup>TM</sup>II HPV28 Detection (Seegene Inc) which detects and  
97 differentiates 28 distinct HPV-types including the 14 hrHPV detected by the Cobas 4800  
98 HPV-test and 14 others HPV (not analyzed in this paper). Both HPV-testing also detect a  
99 human gene as internal control (IC) to assess the quality of the sampling and the efficiency of  
100 the PCR amplification. If IC was not amplified, samples were referred as invalid.

101 Statistical analysis was performed with the package *rstatix* from R software. McNemar and  
102 kappa indices were calculated to compare the proportion of positive samples and the degree of  
103 agreement between the two methods to detect the 14 hrHPV, respectively. A *p*-value of < 0.05  
104 in two-tailed were considered significant.

105

## 106 **RESULTS**

107 Overall, 162 participants were included with a median age of 28.1 [24.4 – 31.8] years,  
108 of whom 56% were MSM and 8% were HIV-infected. None of them received any HPV-  
109 vaccine.

110 One hundred and sixty-two anal samples were tested with both the Roche and Seegene  
111 methods and HPV-testing failed for 59 (36%) and 41 (25%) samples, respectively. The  
112 prevalence of HPV16, HPV18 and others hrHPV types were not significantly different  
113 between the two methods: 20% *versus* 25% (*p*=0.52), 16% *versus* 17% (*p*=0.86) and 50%  
114 *versus* 59% (*p*=0.18), respectively (Table 1).

115 Further statistical analyses for method comparison were performed on 97 samples, 65 samples  
116 being referred as invalid in one or the two methods. The concordance of the results for  
117 HPV16, HPV18 and others hrHPV types detection were 97%, 100% and 88%, respectively  
118 (Table2). Fifteen samples were discordant: (i) 3 for HPV16 positivity, of whom 2 HPV16  
119 missed by Roche and 1 missed by Seegene, (ii) 12 for others hrHPV positivity, of whom 8  
120 hrHPV-types missed by Roche and 4 by Seegene (Supplementary Table 1). HPV52 was the  
121 most common hrHPV type missed by Roche (6/10 cases), followed by HPV16 and HPV56  
122 (2/10 cases each one). For most of the hrHPV missed by Roche, they were detected tardily by  
123 Seegene, after 40 cycles of amplification.

124 The agreement (Cohen's Kappa test) for HPV16, HPV18 and others hrHPV detection  
125 were 0.91 [0.81-1.00], 1.00 [1.00-1.00] and 0.75 [0.62-0.88], respectively. The performance

126 to detect HPV16, HPV18 and others hrHPV positivity were not significantly different  
127 between the 2 tests (McNemar test,  $p>0.05$ ) (Table 2 and Supplementary Table 2). In total,  
128 the global performance to detect any of the 14 hrHPV types was not significantly different  
129 (McNemar test,  $p=0.44$ ).

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## 131 **DISCUSSION**

132 To our knowledge, this is the first study conducted in self-collected anal samples  
133 which compared the Cobas 4800 HPV-test (Roche®) and the Anyplex<sup>TM</sup>II HPV28 Detection  
134 (Seegene Inc) tests for hrHPV detection.

135 First, comparing the 2 methods on 97 samples revealed a good agreement between  
136 them and comparable worldwide ability to identify any of the 14 hrHPV genotypes currently  
137 recommended for the cervical cancer screening. This result was also consistent for HPV16  
138 alone, HPV18 alone and the others hrHPV genotypes detected with the 2 methods. However,  
139 compared to the Seegene approach, the Cobas test missed more anal hrHPV infection. In term  
140 of hrHPV prevalence in normal cervical tissue, a difference was also identified between the  
141 Cobas method and GP5+/6+ PCR testing followed by genotyping with Luminex [4].  
142 However, equal frequency was showed in samples with abnormal cytology. In our work, we  
143 dealt with anal samples and we did not assess the association with cytology results, thus these  
144 two factors may have an impact on the difference between the 2 procedures.

145 The prevalence of HPV16 was the highest in our high-risk population as expected and the 2  
146 methods showed high agreement and performance to detect the most oncogenic genotype in  
147 anal site. This is of importance as HPV16 testing could be one of the biomarkers used as anal  
148 cancer screening in key-population in the near future. For instance in France, new guidelines  
149 propose to use HPV16 testing in anal cytology specimen collected by physician as anal cancer

150 screening marker in MSM living with HIV [9]. If positive, a reflex cytology is performed on  
151 the same sample and patient is referred to a proctologist.

152 Similarly to self-collected vaginal swabs for cervical cancer screening [10], self-collected anal  
153 samples could be of interest to reach patients outside the healthcare system [11]. In our work,  
154 41 (25%) and 59 (36%) samples failed to be amplified by the Anyplex<sup>TM</sup>II HPV28 Detection  
155 and Cobas 4800 HPV-test, respectively. Low-cellularity due to inappropriate sampling and  
156 misunderstanding of the participants may explain this high rate of invalid results. If  
157 implemented in this population, self-collection instructions should be improved.

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159 In conclusion, both the Anyplex<sup>TM</sup>II HPV28 Detection and the Cobas 4800 HPV-test  
160 are suitable to detect the 14 hrHPV genotypes in self-collected anal samples, as well as more  
161 specifically the HPV16 genotype.

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175 **Authors contributions.** Conception and design of the study: AJ, SC and GD; acquisition of  
176 the clinical data: SW, SP and KV; execution of the experiments: GAM; analysis of the data:  
177 AJ, SM and GD; drafting of significant portion of the manuscript or figures: AJ, VC, AGM  
178 and GD. All the authors read, corrected and approved the final version of the manuscript.

179

180 **Transparency declarations.** The authors declare that the research was conducted in the  
181 absence of any commercial or financial relationships that could be construed as a potential  
182 conflict of interest.

183

184 **Ethics.** Permission for the study was obtained from the National Ethics Committee for Health  
185 Research (NECHR) in Cambodia (Approval letter number 189NECHR).

186

187 **Funding.** This study was funded by the Institut Pasteur du Cambodge and the ANRS MIE  
188 (Agence Nationale de recherche sur le SIDA et les hépatites virales - Maladies infectieuses  
189 émergentes).

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231 **Table 1:** Prevalence of HPV16, HPV18 and others hrHPV types found with the 2 assays

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	<b>Seegene N= 121<sup>1</sup></b>	<b>Roche N= 103<sup>2</sup></b>	<i>p-value</i> <sup>3</sup>
<b>HPV16, n (%)</b>			
Negative	91 (75%)	82 (80%)	0.52
Positive	30 (25%)	21 (20%)	
<b>HPV18, n (%)</b>			
Negative	101 (83%)	87 (84%)	0.86
Positive	20 (17%)	16 (16%)	
<b>hrHPV others, n (%)</b>			
Negative	50 (41%)	52 (50%)	0.18
Positive	71 (59%)	51 (50%)	

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234 <sup>1</sup> 41 samples being referred as invalid with Seegene HPV-test

235 <sup>2</sup> 59 samples being referred as invalid with Roche HPV-test

236 <sup>3</sup>Fisher's exact test

237 hrHPV others: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68

238 HPV: human papillomavirus; hrHPV: high-risk human papillomavirus

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255 **Table 2:** Agreement and performance of the two methods to detect HPV16, HPV18 and the  
 256 others hrHPV types  
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	<b>N = 97<sup>1</sup></b>	Cohen's Kappa test	McNemar test
<b>HPV16, n (%)</b>			
Concordant	94 (97%)	0.91 [0.81 - 1.00]	1.00
Discordant	3 (3%)		
<b>HPV18, n (%)</b>			
Concordant	97 (100%)	1.00 [1.00 - 1.00]	NA
Discordant	0 (0%)		
<b>hrHPV others, n (%)</b>			
Concordant	85 (88%)	0.75 [0.62-0.88]	0.39
Discordant	12 (12%)		
<b>All hrHPV, n(%)</b>			
Concordant	90 (93%)	0.85 [0.74–0.96]	0.44
Discordant	7 (7%)		

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259 <sup>1</sup> 65 samples being referred as invalid in one or both HPV-test

260 Cohen's Kappa test and McNemar test were performed to compare the agreement and the performance of the  
 261 two methods to detect HPV16, HPV18, the others hrHPV and the 14 hrHPV as a whole.

262 hrHPV others: HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68

263 HPV: human papillomavirus; hrHPV: high-risk human papillomavirus

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