

**COMPARISON OF FIVE CE/IVD LABELED HPV DNA GENOTYPING ASSAYS****Bozic M, Kessler H H, Koidl C, Marth E***Research Unit Molecular Diagnostics, IHMEM, Medical University of Graz, Austria*

**Objectives:** To compare results obtained by five commercially available CE/IVD labeled assays for detection of HPV DNA in cervical swabs. Accuracies were determined and clinical samples were investigated. Furthermore, the performance in the routine molecular laboratory was evaluated.

**Methods:** For evaluation of accuracies, the Quality Control for Molecular Diagnostics (QCMD) 2009 HPV EQA Pilot study panel ([www.qcmd.org](http://www.qcmd.org)) consisting of 10 members including one negative sample was used. For the clinical study, 138 cervical swabs with ASCUS+ cytological results were collected in Thin Prep Collection vessels (Cytoc Corporation). Results obtained by the LINEAR ARRAY HPV Genotyping Test (Roche), the COBAS 4800 HPV Test (Roche), the Infinity HPV-QUAD (Autogenomics), the AMPLIQUALITY HPV TYPE (AB/Analitica), and the OPEGEN PapillomaStrip (Operon) were compared. All assays were performed with maximum automation for extraction and hybridization/detection. For the cobas 4800 HPV Test, the cobas x 480 was used for extraction and the cobas z 480 for real-time PCR as recommended by the manufacturer. For the remaining assays, DNA extraction was done on the easyMag instrument (Biomerieux). For PCR, the GeneAmp 9700 (Applied Biosystems) was used. Hybridization and detection was done with the ProfiBlot T48 (Tecan) except for the HPV-QUAD assay which was done on the INFINITI Analyser (Autogenomics). Results were considered as valid if the internal control was detected. The LINEAR ARRAY HPV Genotyping Test was taken as reference method. For the performance study, both overall and hands-on times were compared.

**Conclusions:** When the results were compared, 63 samples showed identical and 75 discrepant results. Internal controls were detected throughout the entire study. Overall time required for the different assays ranged from 181 to 465 min, manual time from 20 to 70 min. Because of different detection limits, head to head comparison of different assays may not be conclusive. However, assays evaluated in this study showed reliable results regarding their diagnostic capacities. The clinically relevant threshold was yielded by all assays evaluated in this study. When overall time, manual time, and user-friendliness were compared, considerable differences were observed.

**PERFORMANCE OF COBAS 4800 HPV TEST COMPARED WITH THE HYBRID CAPTURE 2****Pista A., Oliveira A., Verdasca N.***Department of Infectious Diseases, National Institute of Health, Lisbon, Portugal*

**Objective:** To evaluate the performance of the cobas 4800 HPV Test in comparison with the HC2 test in women with cytological and histological results. Further, the clinical performance was evaluated using a clinical cut-off of CIN2+.

**Methods:** The study population comprised 180 archived cervical samples from sexually active women aged 20-65 years old, attending at primary Health-Care Clinics of the National Health Service and Gynaecological Outpatient Clinics that were referred to the National Institute of Health for opportunistic screening and for evaluation of HPV-associated lesions. According to cytology, 33 women had a normal cytology, 45 had ASC-US, 13 had ASC-H, 52 had LSIL, 34 had HSIL, and 3 had SCC. A subset of 160 samples was available for clinical evaluation, based on histological examination of biopsy samples obtained at colposcopy. Of this, 59 were considered to have CIN grade 1 or less ( $\leq$ CIN1, regarded as controls) and 101 were diagnosed as CIN grade 2 or worse ( $\geq$ CIN2, regarded as cases). To assess the reproducibility and the cross-reactivity with LR-HPV genotypes, 13 and 8 samples were retested, respectively. Cases that showed discordant results were subjected to a third test, the CLART Human Papillomavirus 2 assay. All analyses were conducted using the SPSS software.

**Results:** Overall, HR-HPV types were detected in 152 cases (84.4%) with cobas 4800 and in 157 (87.2%) with HC2 test. Analytical and clinical performance of the cobas 4800 Test showed highly comparable outcomes, with very good values of sensitivity (99.0%), PPV, and agreement ( $k=0.795$ ; concordance level 95.0%) compared with the HC2 assay. The specificity (40.7%) and NPV for ?CIN2 was higher by the cobas 4800 Test. In addition, the cobas 4800 Test reproducibility was very good (100%), and no cases of cross-reactivity with other LR-HPV genotypes were observed. Discordant results were observed in 9 samples.

**Conclusions:** The cobas 4800 HPV Test showed an excellent performance for cervical intraepithelial neoplasia grade 2 or worse. The test is efficient, sensitive, reproducible, fully automated, and suitable for large scale testing. Furthermore, this assay has the advantage to concurrently distinguish HPV 16 and 18 from the other HR-HPV genotypes within a single test, which can give more information relative to the predictive value of HR-HPV infection.

# Comparison of five automated CE/IVD labeled HPV DNA genotyping test systems

Michael Bozic (michael.bozic@medunigraz.at), Christoph Koidl, Harald H. Kessler and Egon Marth  
 Research Unit Molecular Diagnostics, IHMEM, Medical University of Graz, Austria

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## Introduction

The human papillomavirus (HPV) has been found to be responsible for precancerous lesions and development of cervical cancer. Several commercial test systems are currently available for direct detection and typing of HPV in cervical swabs; however, manufacturers provide detection of different sets of HPV genotypes. For diagnosis and treatment of suspicious cytological findings in gynecological samples, the clinical sensitivity of the molecular assay used is of maximum importance.

## Aims

In this study, results obtained by five commercially available CE/IVD labeled assays for detection of HPV DNA in cervical swabs were compared. After determination of accuracies, clinical samples were investigated. Furthermore, the performance in the routine molecular laboratory was evaluated.

## Materials and Methods

For evaluation of accuracy, the Quality Control for Molecular Diagnostics (QCMD) 2009 HPV EQA Pilot study panel ([www.qcmd.org](http://www.qcmd.org)) consisting of 10 members including one negative sample was used.

For the clinical study, 138 cervical swabs with confirmed ASCUS+ cytological results were collected in Thin Prep Collection vessels (Cytoc Corporation). Results obtained by the LINEAR ARRAY HPV Genotyping Test (Roche), the COBAS 4800 HPV Test (Roche), the Inifinity HPV-QUAD (Bülmann), the AMPLIQUALITY HPV TYPE (ABAnalytica), and the OPEGEN PapillomaStrip (Operon) were compared. Results were considered as valid if the internal control was detected. The LINEAR ARRAY HPV Genotyping Test was taken as reference method. All assays were performed with a maximum degree of automation for each step (extraction, hybridization, and detection). DNA extraction was done on the EasyMag instrument (Biomerieux). For PCR, the Gene Amp 9700 (Applied Biosystems) was used. Hybridization and detection was done with the ProBiotM 48 (Tecan). For the COBAS 4800 HPV Test, the cobas x480 instrument was used for extraction and the cobas z 480 for PCR and detection as recommended by the manufacturer.

For the performance study, both the overall and hands-on-times were compared.

## Results

Results of accuracy testing are shown in Table 1. According to the guidelines for the early detection of cervical cancer in patients with abnormal cytopathology using an HPV triage assay, the distributor considered panel samples containing HPV copies below the clinically-relevant threshold as determined by the FDA-approved HC2 test as clinically negative (HPV DNA in RV4, RV9, RV10). All results obtained by assays used in this study were thus considered as correct.

When comparing the results obtained from cervical swabs, 63 samples showed identical and 75 discrepant results (Table 2). Internal controls were detected throughout the whole entire study.

Overall time required for the different assays ranged from 181 to 465 min (Table 3). Manual time required for the different assays ranged from 20 to 70 min.

Table 1. Results obtained from accuracy testing.

HPV genotype	HPV genotype control	Linear array (Roche)	HPV-QUAD (Bülmann)	COBAS 4800 (Roche)	AMPLIQUALITY HPV TYPE (ABAnalytica)	OPEGEN PapillomaStrip (Operon)
RV2	16	10000	16	16	16	16
RV4	18	10000	16	16	16	16
RV6	18	10000	16	16	16	16
RV8	18	10000	16	16	16	16
RV9	18	10000	16	16	16	16
RV10	18	10000	16	16	16	16
RV11	18	10000	16	16	16	16
RV12	18	10000	16	16	16	16
RV13	18	10000	16	16	16	16
RV14	18	10000	16	16	16	16
RV15	18	10000	16	16	16	16
RV16	18	10000	16	16	16	16
RV17	18	10000	16	16	16	16
RV18	18	10000	16	16	16	16
RV19	18	10000	16	16	16	16
RV20	18	10000	16	16	16	16
RV21	18	10000	16	16	16	16
RV22	18	10000	16	16	16	16
RV23	18	10000	16	16	16	16
RV24	18	10000	16	16	16	16
RV25	18	10000	16	16	16	16
RV26	18	10000	16	16	16	16
RV27	18	10000	16	16	16	16
RV28	18	10000	16	16	16	16
RV29	18	10000	16	16	16	16
RV30	18	10000	16	16	16	16
RV31	18	10000	16	16	16	16
RV32	18	10000	16	16	16	16
RV33	18	10000	16	16	16	16
RV34	18	10000	16	16	16	16
RV35	18	10000	16	16	16	16
RV36	18	10000	16	16	16	16
RV37	18	10000	16	16	16	16
RV38	18	10000	16	16	16	16
RV39	18	10000	16	16	16	16
RV40	18	10000	16	16	16	16
RV41	18	10000	16	16	16	16
RV42	18	10000	16	16	16	16
RV43	18	10000	16	16	16	16
RV44	18	10000	16	16	16	16
RV45	18	10000	16	16	16	16
RV46	18	10000	16	16	16	16
RV47	18	10000	16	16	16	16
RV48	18	10000	16	16	16	16
RV49	18	10000	16	16	16	16
RV50	18	10000	16	16	16	16
RV51	18	10000	16	16	16	16
RV52	18	10000	16	16	16	16
RV53	18	10000	16	16	16	16
RV54	18	10000	16	16	16	16
RV55	18	10000	16	16	16	16
RV56	18	10000	16	16	16	16
RV57	18	10000	16	16	16	16
RV58	18	10000	16	16	16	16
RV59	18	10000	16	16	16	16
RV60	18	10000	16	16	16	16
RV61	18	10000	16	16	16	16
RV62	18	10000	16	16	16	16
RV63	18	10000	16	16	16	16
RV64	18	10000	16	16	16	16
RV65	18	10000	16	16	16	16
RV66	18	10000	16	16	16	16
RV67	18	10000	16	16	16	16
RV68	18	10000	16	16	16	16
RV69	18	10000	16	16	16	16
RV70	18	10000	16	16	16	16
RV71	18	10000	16	16	16	16
RV72	18	10000	16	16	16	16
RV73	18	10000	16	16	16	16
RV74	18	10000	16	16	16	16
RV75	18	10000	16	16	16	16
RV76	18	10000	16	16	16	16
RV77	18	10000	16	16	16	16
RV78	18	10000	16	16	16	16
RV79	18	10000	16	16	16	16
RV80	18	10000	16	16	16	16
RV81	18	10000	16	16	16	16
RV82	18	10000	16	16	16	16
RV83	18	10000	16	16	16	16
RV84	18	10000	16	16	16	16
RV85	18	10000	16	16	16	16
RV86	18	10000	16	16	16	16
RV87	18	10000	16	16	16	16
RV88	18	10000	16	16	16	16
RV89	18	10000	16	16	16	16
RV90	18	10000	16	16	16	16
RV91	18	10000	16	16	16	16
RV92	18	10000	16	16	16	16
RV93	18	10000	16	16	16	16
RV94	18	10000	16	16	16	16
RV95	18	10000	16	16	16	16
RV96	18	10000	16	16	16	16
RV97	18	10000	16	16	16	16
RV98	18	10000	16	16	16	16
RV99	18	10000	16	16	16	16
RV100	18	10000	16	16	16	16

Table 3. Overall times required for HPV assays.

Assay	Linear array HPV (Roche)	HPV-QUAD (Bülmann)	COBAS 4800 (Roche)	AMPLIQUALITY HPV TYPE (ABAnalytica)	OPEGEN PapillomaStrip (Operon)
Sample preparation	10 min	10 min	10 min	10 min	10 min
Extraction	Mediate EasyMag Biomixer 50 min	Mediate EasyMag Biomixer 50 min	Mediate EasyMag Biomixer 50 min	Mediate EasyMag Biomixer 50 min	Mediate EasyMag Biomixer 50 min
Amplification	100 min	100 min	100 min	100 min	100 min
Sample preparation	140 min	200 min	90 min	20 min	120 min
Analysis	10 min	470 min	5 min	10 min	10 min
Total	420 min	770 min	181 min	395 min	300 min

Table 2. Discrepant results.

Number	Linear array HPV	Inifinity HPV-QUAD	HPV-QUAD 4800	Roche	Operon
1	51,58,92	30,45,92,93,91	16	16	16
2	16	16	16	16	16
3	16	16	16	16	16
4	16	16	16	16	16
5	16	16	16	16	16
6	16	16	16	16	16
7	16	16	16	16	16
8	16	16	16	16	16
9	16	16	16	16	16
10	16	16	16	16	16
11	16	16	16	16	16
12	16	16	16	16	16
13	16	16	16	16	16
14	16	16	16	16	16
15	16	16	16	16	16
16	16	16	16	16	16
17	16	16	16	16	16
18	16	16	16	16	16
19	16	16	16	16	16
20	16	16	16	16	16
21	16	16	16	16	16
22	16	16	16	16	16
23	16	16	16	16	16
24	16	16	16	16	16
25	16	16	16	16	16
26	16	16	16	16	16
27	16	16	16	16	16
28	16	16	16	16	16
29	16	16	16	16	16
30	16	16	16	16	16
31	16	16	16	16	16
32	16	16	16	16	16
33	16	16	16	16	16
34	16	16	16	16	16
35	16	16	16	16	16
36	16	16	16	16	16
37	16	16	16	16	16
38	16	16	16	16	16
39	16	16	16	16	16
40	16	16	16	16	16
41	16	16	16	16	16
42	16	16	16	16	16
43	16	16	16	16	16
44	16	16	16	16	16
45	16	16	16	16	16
46	16	16	16	16	16
47	16	16	16	16	16
48	16	16	16	16	16
49	16	16	16	16	16
50	16	16	16	16	16
51	16	16	16	16	16
52	16	16	16	16	16
53	16	16	16	16	16
54	16	16	16	16	16
55	16	16	16	16	16
56	16	16	16	16	16
57	16	16	16	16	16
58	16	16	16	16	16
59	16	16	16	16	16
60	16	16	16	16	16
61	16	16	16	16	16
62	16	16	16	16	16
63	16	16	16	16	16
64	16	16	16	16	16
65	16	16	16	16	16
66	16	16	16	16	16
67	16	16	16	16	16
68	16	16	16	16	16
69	16	16	16	16	16
70	16	16	16	16	16
71	16	16	16	16	16
72	16	16	16	16	16
73	16	16	16	16	16
74	16	16	16	16	16
75	16	16	16	16	16
76	16	16	16	16	16
77	16	16	16	16	16
78	16	16	16	16	16
79	16	16	16	16	16
80	16	16	16	16	16
81	16	16	16	16	16
82					

