Comparison of the Hybrid Capture 2 assay and PapilloCheck HPV-Screening tests for detection of high-risk HPV genotypes in genital samples.

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INTRODUCTION

Human papillomavirus (HPV) is a common sexually transmitted disease caused by a large group of viruses that infect the skin and genital mucosa of humans, often causing irregular cell growth and warts. Persistent HPV infection may lead to cervical cancer in some cases. Progression of disease depends on infection caused by certain types of HPV, called high-risk types (HR-HPV). Others are more often associated with benign warts and belong to the low-risk group (LR-HPV).

HR-HPV: 16,18,26,31,33,35,39,45,51,52,53,56,58,59,66,68,70,73,82
LR-HPV: 6,11,42,43,44,54,55,57,61,62,64,67,69,70,71,72,81,83

Together, HPV-16 and HPV-18 are responsible for 70% of all cases of cervical cancers.

Several molecular assays have been developed for HPV screening in genital samples. The Digene Hybrid Capture 2 (HC2) assay has largely been used to detect the presence of 13 high-risk human papillomaviruses types in Brazil. Recently, other methodologies became available to detect and differentiate among the various HPV types involved in genital infections.

OBJECTIVE

To compared the agreement of HPV detection using the Hybrid Capture 2 and PapilloCheck screening test, a PCR-based test coupled with a rapid hybridization step.

MATERIAL AND METHODS

We compared 93 genital samples collected using the Universal Collection Medium (Digene) by both methods. Samples were collected and processed by HC2 to detect high-risk HPV types only, following the manufacturer’s instructions. For PC screening, after being used for HC2 test, viral DNA was extracted from denatured samples (1 mL) using the Magna Pure LC DNA Isolation Kit (large volumes) and the Magna Pure LC platform (Roche). The isolated DNA was subjected to PCR amplification, prior to hybridization on the Microarray products. The hybridization DNA probes fixed on the Papillocheck DNA array. After hybridization, the DNA arrays were automatically analyzed using the Check Scanner (Greiner Bio One). The PC test permitted to identify the HPV genotype(s) present in each sample.

Conventional (MV 09/11) and/or real-time PCR (Payan et al) were used a resolve discordant results. For comparison, only samples that were considered positive for HR-HPV were included in the statistical analysis.

The software EP Evaluator 7.0 was used to determine the agreement between the tests.

PapilloCheck: Test procedures

DNA Extraction (1 h) → PCR (2.5 h) → Hybridization (15 min) → Scanning (10 min)

RESULTS

FIG. 1: Comparison of HC2 and PapilloCheck screening tests

<table>
<thead>
<tr>
<th>Statistical Summary</th>
<th>Reference Method: PC</th>
<th>Test Method: HC2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive Reference</td>
<td>Negative Reference</td>
</tr>
<tr>
<td>Positive Test</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>Negative Test</td>
<td>9</td>
<td>79</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>71</td>
</tr>
</tbody>
</table>

FIG. 2: Prevalence of HPV genotypes identified by PapilloCheck test

FIG. 3: Occurrence of Single vs. Multiple Subtypes among Positive Samples

CONCLUSIONS

1. Overall, the agreement was 89.2%, with 88.7% and 90.7% of positive and negative agreement, respectively. The Cohen’s kappa coefficient was 72.8%.
2. Eight samples were positive by PC and CH2 negative. In all cases a high-risk HPV type was detected, including 2 with HPV-18. These samples were considered false-negative by CH2.
3. Two samples were positive by CH2 and negative by PC. Both samples had low RLU values and were negative by a third PCR reaction. These were considered false-positive by HC2.
4. PapilloCheck permitted to know the HPV types involved in these infections. Several subtypes other than 16 and 18 were found in relatively high proportion of patients.
5. Almost 60% of our patients were infected with multiple subtypes. The significance of that finding is still under debate, but it seems that coinfection increases the risk for progression to cervical cancer and invasive diseases.
6. Genotyping may be an important tool for patient management, since only patients with persistent infections by the same HPV subtype are at risk of cervical cancer development.

REFERENCES