Validation of an automated detection platform
for use with the Roche Linear Array HPV Genotyping Test

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Short Title: Automated detection for the HPV Linear Array

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ABSTRACT

An automated platform (BeeBlot) was evaluated in parallel with the recommended protocol for hybridization and detection steps of the Roche Linear Array HPV genotyping test using DNA from 143 cervical specimens. Genotyping profiles showed 100% concordance between methods, suggesting automation could complement the Linear Array for enhanced speed and reproducibility.
Infection with high-risk (HR) human papillomavirus (HPV) genotypes is a major causative factor for developing cervical cancer and its precursor lesions (2, 3, 13, 14, 22). There are approximately 40 HPV genotypes known to infect the human anogenital mucosa, which are divided into low-risk (LR) and HR types based on their implicated etiology in cervical carcinoma (8, 15). Infections with either HPV risk type may result in abnormal cell growth, though most are transient, asymptomatic and spontaneously cleared by the immune system. However, persistent infection with HR-HPV genotypes is a significant risk factor in the progression of cervical lesions from low-grade into high-grade and potentially carcinoma of the cervix (11, 23).

Molecular techniques for HPV detection are widely used, with PCR-based assays providing a sensitive and non-invasive approach for monitoring the presence of active HPV infections (5, 6, 16, 17). Accurate identification of HPV genotypes is important for epidemiological studies, including monitoring persistent HR-HPV infections. The Linear Array® HPV Genotyping Test (LA-HPV) (Roche Diagnostics) offers a reliable, sensitive and standardized approach for detecting HPV DNA in cervical specimens (4, 7, 18, 21). The LA-HPV is a qualitative in vitro PCR-based test allowing the detection of up to 37 anogenital HPV genotypes, including the major HR types (7, 10). HPV genotyping has important clinical applications: evaluating clearance and re-infection of specific HPV types; monitoring treatment success for high-grade cervical disease; determining HPV-type prevalence in different populations for pre- and post-evaluation of prophylactic HPV vaccine impact (1, 17). The LA-HPV is a highly standardized assay, including reagents, amplification profiles, and hybridization and detection conditions, for optimal sensitivity and reproducibility. The test comprises four main processes: DNA extraction; PCR
amplification of target sequences; hybridization of PCR-product to specific oligonucleotide probes on a nylon strip; and colorimetric detection (4, 7, 9, 18, 21). The recommended protocol for hybridization and detection involves a labor-intensive and time-consuming procedure, which could potentially cause varied reproducibility. With the aim of reducing labor-intensiveness of the LA-HPV assay, we evaluated the BeeBlot automated platform as an alternative method for the LA-HPV hybridization and detection steps.

Cervical brush specimens \((n = 143)\) were selected, from a cohort of 1,679 specimens with varying Hybrid Capture 2 results (comprising 68 negative and 75 positive) to assess genotyping sensitivity using extracts with low to high HPV viral load. All specimens were collected in PreservCyt (Cytyc Corporation), between May 2001 and December 2002, from women undergoing ablative treatment for histologically confirmed cervical abnormality at the Royal Women’s Hospital, Melbourne, Australia.

DNA was extracted from specimens using the MagNA Pure LC system with a modified procedure, as previously described (18). In brief, a 1 ml aliquot was pelleted and resuspended into 200 µl sterile PBS and extracted using the DNA-I protocol into 100 µl. DNA was genotyped using the reverse line-blot LA-HPV test. PCR was performed in a 100 µl volume, using 50 µl LA-HPV master mix (Roche Molecular Systems) and 50 µl DNA template, as previously described (18, 20). Seventy five µl of the same denatured PCR product was detected using both protocols; air-incubator manual method (19) and BeeBlot automated method, ensuring an accurate comparison.

The BeeBlot (Bee Robotics Ltd, Gwynedd, UK) is a fully automated platform for washing and hybridization steps required by strip-based assays, such as the LA-HPV. All
reagents were prepared immediately prior to each run. Two reagent priming steps and a
preheat (51.5°C) were performed prior to each detection run. Comparison of the
incubation and turnaround times for each method is summarized in Table 1.

Assessing whether positioning within the BeeBlot tray affected hybridization
efficiency (including reproducibility of signal intensity), six specimens with multiple
HPV genotypes were amplified then hybridized at 53°C in three positions across the tray
(left, centre and right). HPV and β-globin signal intensities decreased from the left-hand
side of the tray to the right-hand side at a hybridization temperature of 53°C (Figure 1A),
thought to be the result of a 2°C temperature differential identified across the tray, which
is within the BeeBlot operational specifications (Jones, S. – Bee Robotics, personal
communication). To reduce signal disparity across the tray, subsequent hybridization (and
stringent washing) were performed at 51.5°C, with signal reproducibility markedly
improving across the tray (Figure 1B).

Following initial BeeBlot validation, 143 specimens were assessed for a more
comprehensive evaluation. Of the 143 DNA extracts, one tested negative for β-globin and
HPV by both detection methods and removed from the analysis. Collectively, specimen
adequacy was 99.3% (142/143). Comparing resultant HPV genotyping profiles, a
concordance of 100% (142/142) (κ = 1.0) was observed. Levels of background and signal
intensities varied marginally between detection methods, with the manual approach
having slightly higher signal intensity levels, as well as a minor increase in background.
A sample comparison of 15 HPV strips is provided in Figure 2. Genotyping profiles of
the 143 specimens ranged from single HPV infections to multiple HPV infections, with
up to 7 HPV genotypes detected (Table 2). Approximately one third of the specimens
contained single HPV infections (31.7%), with 29.6% HPV-negative and 38.7% containing multiple genotypes. These findings corroborate the equivalent performance between the manual and automated detection protocols for identifying varying quantities of HPV genotypes among clinical specimens.

The recently released LA-HPV genotyping test provides a standardized, consistent, and rapid means for identifying HPV genotypes within clinical specimens. This permits the assessment of whether persistence of a specific HPV genotype is the basis of recurrent HPV positivity, thus denoting a substantially increased risk of cervical disease progression (11, 22, 23). Although HPV type persistence can be assessed by LA-HPV, there is currently no standardized recommendation for using genotype persistence on patient management. The LA-HPV hybridization and detection steps can be considered labor-intensive and time-consuming, particularly for extensive genotyping studies. Incorporating automation into these steps would greatly facilitate the HPV genotyping test, providing simplicity, improving time and labor efficiency and most importantly, accuracy and reproducibility of results.

The BeeBlot, as an automated processing platform for use with the LA-HPV test, was evaluated and validated in this study. This platform can accommodate from 2 to 48 samples (in multiples of two), with a full run of 48 DNA extracts typically genotyped within a 2½ h period. Signal intensities across the plate (for both HPV and β-globin) were most consistent when hybridization and stringent wash steps were performed at 51.5°C, which is imperative for assay reproducibility. Among 142 valid specimens, HPV genotyping profiles obtained were identical using either the manual or automated procedure (concordance of 100%). To further improve the consistency and
reproducibility of the Linear Array genotyping test, particularly during interpretation of HPV bands, use of a scanner or other such automated device to quantify band intensities would prove highly beneficial, as recently reported (12).

In conclusion, these findings indicate that the BeeBlot automated platform, as a supplementary tool to the LA-HPV test, has a capacity equal in sensitivity to the current recommended detection protocol, for typing single and multiple HPV infections. Laboratories, particularly involved in large-scale HPV genotyping studies, would find automated platforms, such as the BeeBlot, more simplified, less time-consuming and potentially more reproducible than the recommended manual detection approach. With these findings, the BeeBlot automated hybridization and detection system could quite effectively be utilized for processing LA-HPV strips upon appropriate internal laboratory validation. Other automated hybridization and detection platforms for strip-based assays; such as the ProfitBlot (Tecan Group Ltd), Genelabs AutoBlot 20/36 systems (Genelabs Diagnostics) and MedTec’s AutoBlot 2000/6000 processors (Helvatica Health Care) provide similar advantages, though also require validation prior to implementation in the laboratory.

We would like to thank Roche Molecular Systems for providing the Linear Array HPV genotyping and detection kits enabling the completion of this study.

REFERENCES


Figure Legends:

FIG. 1. BeeBlot detection of LA-HPV strips using hybridization and stringent wash temperatures of 53°C or 51.5°C. Strips shown are those detected in the far left ‘L’ and far right ‘R’ 6 positions of the BeeBlot tray, as indicated on the strip label.

FIG. 2. Comparison of manual and BeeBlot LA-HPV detection. Fifteen specimens with varying HPV-type profiles, detected by both methods, are shown. HPV strips on the left are those detected by the manual method, while those on the right were detected using the BeeBlot.
**TABLE 1.** Comparison of incubation and turnaround times for the manual versus automated detection protocols.

<table>
<thead>
<tr>
<th>Step</th>
<th>Manual protocol (24 tests)</th>
<th>BeeBlot protocol $^a$ (48 tests)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (min)</td>
<td>Incubation temp ($^0$C)</td>
</tr>
<tr>
<td>Hybridization</td>
<td>30</td>
<td>53</td>
</tr>
<tr>
<td>Ambient Wash</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Stringent Wash</td>
<td>15</td>
<td>53</td>
</tr>
<tr>
<td>Conjugate</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Ambient Wash</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ambient Wash</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
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<tr>
<td></td>
<td>5</td>
<td></td>
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<tr>
<td>Ambient Wash</td>
<td>5</td>
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<td>5</td>
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<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Incubation time</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>Turnaround time</td>
<td>180 (approx)</td>
<td></td>
</tr>
</tbody>
</table>

Buffer preparation and strip labeling times are similar for both methods. $^a$ Times and incubation temperatures as entered into the BeeBlot instrument. $^b$ Strips can be labelled during the pre-heat incubation. $^c$ Turnaround time includes additional hands-on and/or instrument processing times.
**Table 2.** Number of HPV genotypes per specimen detected: manual versus automated detection.

<table>
<thead>
<tr>
<th>Number of HPV types detected</th>
<th>No. of specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative $^a$</td>
<td>42 (29.6)</td>
</tr>
<tr>
<td>1</td>
<td>45 (31.7)</td>
</tr>
<tr>
<td>2</td>
<td>22 (15.5)</td>
</tr>
<tr>
<td>3</td>
<td>11 (7.8)</td>
</tr>
<tr>
<td>4</td>
<td>9 (6.3)</td>
</tr>
<tr>
<td>5</td>
<td>6 (4.2)</td>
</tr>
<tr>
<td>6</td>
<td>4 (2.8)</td>
</tr>
<tr>
<td>7</td>
<td>3 (2.1)</td>
</tr>
</tbody>
</table>

One specimen negative for both β-globin and HPV was excluded. $^a$ A negative result refers to HPV negativity as per LA-HPV test.
Figure 1.
Figure 2.

β-globin

HPV bands

Reference line