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Validation of an Automated Detection Platform for Use with the Roche Linear Array Human Papillomavirus Genotyping Test[▽]

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An automated platform (BeeBlot) was evaluated in parallel with the recommended protocol for the hybridization and detection steps of the Roche Linear Array human papillomavirus (HPV) genotyping test using DNA from 143 cervical specimens. Genotyping profiles showed 100% concordance between the methods, suggesting that automation could complement the Roche Linear Array for enhanced speed and reproducibility.

Infection with high-risk (HR) human papillomavirus (HPV) genotypes is a major causative factor for development of 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 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 to carcinoma of the cervix (11, 23).

Molecular techniques for HPV detection are widely used, with PCR-based assays providing a sensitive and noninvasive 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 (LA-HPV) genotyping test (Roche Diagnostics) offers a reliable, sensitive, and standardized approach for detecting HPV DNA in cervical specimens (4, 7, 18, 21). The LA-HPV test 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 reinfection of specific HPV types, monitoring treatment success for high-grade cervical disease, and determining HPV type prevalence in different populations for pre- and postevaluation of prophylactic HPV vaccine impact (1, 17). The LA-HPV test 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 products to specific oligonulceotide 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 the 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 different Hybrid Capture 2 results (68 negative and 75 positive specimens were selected) to assess genotyping sensitivity using extracts with low to high HPV viral loads. 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, resuspended in 200 μl sterile phosphate-buffered saline, 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 microliters of the same denatured PCR product was detected using both protocols, i.e., the air incubator manual method (19) and the BeeBlot automated method, ensuring an accurate comparison.

The BeeBlot (Bee Robotics Ltd., Gwynedd, United Kingdom) is a fully automated platform for the 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 preheating (51.5°C) were performed prior to each detection run. A comparison of the incubation and turnaround times for the two methods is summarized in Table 1.

To assess whether positioning within the BeeBlot tray affected hybridization efficiency (including reproducibility of signal intensity), six specimens with multiple HPV genotypes were amplified and then hybridized at 53°C in three positions across the tray (left, center, and right). HPV and β-globin signal intensities decreased from the left side of the tray to the right side at a hybridization temperature of 53°C (Fig. 1A); this was thought to be the result of a 2°C temperature differential

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TABLE 1. Comparison of incubation and turnaround times for the manual and automated detection protocols

Protocol (no. of tests)	Step	Time (min)[a]	Incubation temp (°C)
Manual (24)	Hybridization	30	53
	Ambient wash	1	
	Stringent wash	15	53
	Conjugate	30	
	Ambient wash	1	
	Ambient wash	10	
	Ambient wash	10	
	Ambient wash	5	
	Citrate	5	
	Substrate	10	
	Distilled water	5	
	Distilled water	5	
	Distilled water	0	
	Incubation time	127	
	Turnaround time[b]	180 (approx)	
BeeBlot (48)[c]	Preheat	15[d]	51.5
	Hybridization	30	51.5
	Ambient wash	0	
	Conjugate	15	
	Stringent wash	12	51.5
	Ambient wash aspirate	0	
	Ambient wash dispense	5	
	Ambient wash	5	
	Citrate	5	
	Substrate	10	
	Distilled water	0	
	Distilled water	0	
	Distilled water	0	
	Incubation time	97	
	Turnaround time	150 (approx)	

[a] Buffer preparation and strip labeling times are similar for both methods.
[b] Turnaround time includes additional hands-on and/or instrument processing times.
[c] Times and incubation temperatures as entered into the BeeBlot instrument.
[d] Strips can be labeled during the preheat incubation.

identified across the tray, which is within the BeeBlot operational specifications (S. Jones, Bee Robotics, personal communication). To reduce the signal disparity across the tray, subsequent hybridizations (and stringent washing) were performed at 51.5°C, with signal reproducibility markedly improving across the tray (Fig. 1B).

Following the 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 were 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 the 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 Fig. 2. Genotyping profiles of the 143 specimens ranged from single HPV infections to multiple HPV infections, with up to seven HPV genotypes detected (Table 2). Approximately one-third of the specimens contained single HPV infections (31.7%), with 29.6% being HPV negative and 38.7% containing multiple genotypes. These findings corroborate the equivalent performances of the manual and automated detection protocols in identifying various 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 with the LA-HPV test, there is currently no standardized recommendation for using genotype persistence for 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

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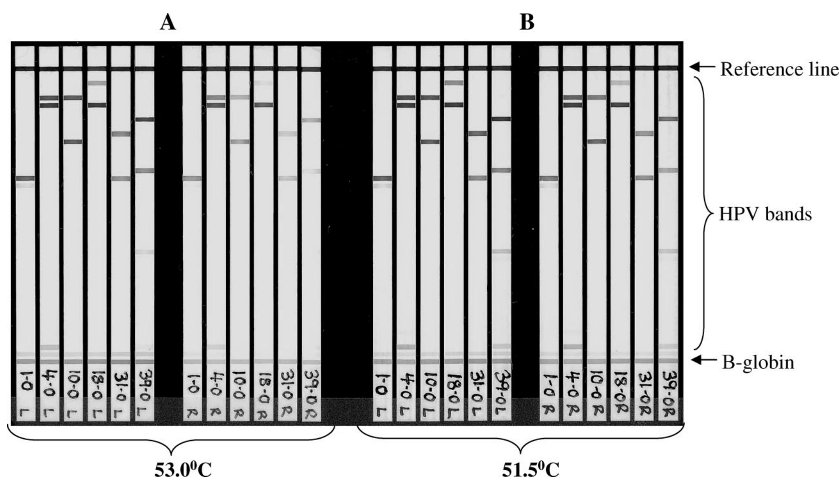

FIG. 1. BeeBlot detection of LA-HPV strips using hybridization and stringent wash temperatures of 53°C or 51.5°C. The strips shown are those detected in the far left (L) and far right (R) six positions of the BeeBlot tray, as indicated on the strip label.

FIG. 2. Comparison of manual and BeeBlot LA-HPV detection. Fifteen specimens with various 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.

simplicity and improving time and labor efficiency and, most importantly, the 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 2), with a full run of 48 DNA extracts typically genotyped within a 2 1/2-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, the 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 LA-HPV genotyping test, particularly during interpretation of HPV bands, the use of a scanner or other such automated device to quantify band intensities would be highly beneficial, as recently reported (12).

In conclusion, these findings indicate that the BeeBlot automated platform, as a supplementary tool with 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 those involved in large-scale HPV genotyping studies, would find automated platforms, such as the BeeBlot, simpler, 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 (Helvetica Health Care), provide similar advantages, though they also require validation prior to implementation in the laboratory.

TABLE 2. Number of HPV genotypes per specimen detected by manual versus automated detection[a]

No. of HPV types detected	No. (%) of specimens
0[b]	42 (29.6)
1	45 (31.7)
2	22 (15.5)
3	11 (7.8)
4	9 (6.3)
5	6 (4.2)
6	4 (2.8)
7	3 (2.1)

[a] One specimen, which was negative for both β-globin and HPV, was excluded.
[b] HPV negativity per the LA-HPV test.

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