Human Papillomavirus Genotyping Using Archival Vulval Dysplastic or Neoplastic Biopsy Tissues: Comparison between the INNO-LiPA and Linear Array Assays

Sarah E. Tan,1,2,* Suzanne M. Garland,1,2,3 Alice R. Rumbold,4,5 and Sepehr N. Tabrizi1,2,3

Department of Microbiology and Infectious Diseases, The Royal Women’s Hospital, Parkville, Victoria, Australia1; Department of Obstetrics and Gynaecology, the University of Melbourne, Parkville, Victoria, Australia2; Murdoch Children’s Research Institute, Parkville, Victoria, Australia3; Services, Systems, and Society Division, Menzies School of Health Research, Casuarina, Northern Territory, Australia4; and Discipline of Obstetrics and Gynaecology, the University of Adelaide, Adelaide, South Australia, Australia5

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The Roche Linear Array (LA) and Innogenetics INNO-LiPA human papillomavirus (HPV) genotyping assays were compared for paraffin-embedded vulval tissues. The LA detected amplifiable DNA in 28 (57%) out of 49 biopsy specimens, 20 (40%) being HPV genotyped, compared to 49 (100%) and 41 (83%), respectively, detected by the INNO-LiPA. The INNO-LiPA provides greater sensitivity for HPV genotyping in archival tissue.

Persistent infection with oncogenic human papillomavirus (HPV) is a prerequisite for the development of HPV-related neoplastic precancerous changes in the anogenital region (13). Cervical and vulval biopsy specimens embedded in paraffin for histological diagnoses can also be utilized for detection of HPV genotypes. However, sensitive and accurate detection of HPV genotypes in such archival tissues could be affected, as DNA is often degraded as a result of long and poor storage conditions. Such damage to DNA includes chemical modification, cross-linking, and fragmentation, all of which can reduce the efficiency of PCR amplification (1, 6).

This study compared two commercial HPV genotyping assays: the Linear Array assay (LA) (Roche Molecular Systems, Alameda, CA) (2) and the INNO-LiPA Genotyping Extra assay (LiPA) (Innogenetics, Ghent, Belgium) (5) to detect and type HPV in paraffin-embedded vulval tissue biopsy specimens. Although both methods have been applied previously to paraffin-embedded tissues (5, 7), a comparison of the two assays, to our knowledge, has not been reported to date.

The paraffin-embedded biopsy specimens were from a total of 49 histologically diagnosed cases of vulval disease, of which 30 were high-grade vulval intraepithelial neoplasia (VIN grade 2/3) and 19 were invasive vulvar cancer, evaluated and diagnosed between January 1996 and December 2005 in women residing in the Northern Territory of Australia.

A sandwich sectioning method was used, with the outer sections stained with hematoxylin and eosin to confirm the histological diagnosis of the inner sections used for HPV detection (4). To minimize cross contamination, the microtome blade was changed and the microtome surface was cleaned after each sample was sectioned. Gloves were changed regularly, and the sectioning of control HPV-negative tissue specimens was carried out in a random order to ensure no possibility of HPV DNA carryover between sectioning of samples.

Each specimen was sectioned and deparaffinized according to the manufacturer’s instructions for a Roche DNA Isolation tissue kit (Roche Molecular Systems). Briefly, a 7-μm section was mixed with a 1.2-ml mixture of histolene (800 μl) and ethanol (400 μl). The tissue was pelleted at 14,000 × g for 10 min, and the resultant pellet washed twice with 500 μl of 70% ethanol. The tissue was air dried to ensure that no residual ethanol was present and was subsequently incubated with 160 μl of tissue lysis buffer (Roche Molecular Systems) and 40 μl of proteinase K (Roche Molecular Systems) on a 55°C heat block until fully digested. The digested tissue was then transferred onto an automated Roche MagNA Pure LC isolation and purification system, using a DNA Isolation kit 1. The final isolated DNA volume in elution buffer was 100 μl.

The Roche Linear Array HPV genotyping test (Roche Molecular Systems) directs the amplification of a 450-bp region of the HPV L1 gene and allows the identification of 37 anogenital HPV genotypes: HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, and 83.

### Table 1. Numbers of assessable samples that were internal control positive and/or genotype positive, internal control positive only, or internal control negative and HPV genotype positive in LiPA and LA

| Result | LiPA | LA |
|--------|------|----|
| Internal control and/or genotype positive | 49 | 28* |
| Internal control only positive | 49 | 20 |
| Internal control negative and HPV genotype positive | 0 | 8 |

* Corresponding author. Mailing address: Department of Microbiology and Infectious Diseases, The Royal Women’s Hospital, Locked Bag 300, Parkville 3052, Australia. Phone: (61-3) 8345-3678. Fax: (61-3) 8345-2225. E-mail: s.tan39@pgrad.unimelb.edu.au.

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*Corresponding author. Mailing address: Department of Microbiology and Infectious Diseases, The Royal Women’s Hospital, Locked Bag 300, Parkville 3052, Australia. Phone: (61-3) 8345-3678. Fax: (61-3) 8345-2225. E-mail: s.tan39@pgrad.unimelb.edu.au.

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TABLE 2. Comparison of the detection of single HPV infections by the assays

| HPV genotype | No. of samples with genotype found by: |
|--------------|--------------------------------------|
|              | LiPA | LA |
| 16           | 28   | 17$^a$ |
| 26           | 1    | 0   |
| 31           | 1    | 0   |
| 33           | 2    | 1   |
| 35           | 1    | 0   |
| 39           | 1    | 0   |
| Total        | 34   | 18  |

$^a$ P = 0.0258.

TABLE 3. Comparison of the detection of multiple HPV infections by the assays

| Biopsy specimen | HPV genotype(s) found by: |
|-----------------|----------------------------|
| LiPA            | LA |
| 1               | 16, 66 | 16 |
| 2               | 66, 82 | None |
| 3               | 16, 56 | 56 |
| 4               | 16, 59 | 16, 59 |
| 5               | 51, 82 | None |
| 6               | 11, 35 | 84$^b$ |
| 7               | 16    | 6, 16 |
| 8               | 16, 52 | 16, 84$^b$ |

$^a$ The total numbers of samples with multiple infections found by the assays were 7 for the LiPA and 3 for the LA. Of a total of 49 samples, the LiPA found 41 to be HPV positive and the LA found 20 to be HPV positive. $^b$ HPV 84 is only detected by the LA.

result in decreased sensitivity on paraffin-embedded tissue. A previous study demonstrated the use of the LA on archival cervical cancer tissue (7); however, this method involved screening samples with primers targeting the HPV L1 gene and utilized a nested PCR approach for application with the LA. A nested approach is time consuming and more prone to contamination and hence is not practical for large epidemiological studies. The LiPA has a greater sensitivity for detection, given the smaller amplicon target, and so a greater number of samples will produce assessable results, as found in this study. Similar to other PCR assays, it also has the limitation of detecting not only the agent causing a particular lesion (one genotype per lesion, since they are clonal) but other colonizing HPVs, related but not causing the lesion (12).

As tissue biopsy specimens are routinely embedded in paraffin for histological diagnoses and then stored over time, there is an important need for accurate detection of HPV genotypes in archival tissue. Although a limitation of this study is the small number of samples examined, the LiPA assay had greater sensitivity than did the LA in the detection of HPV genotypes in paraffin-embedded tissue. Although the LA is able to provide accurate results for liquid-based cytology (2, 11) and frozen tissue (14), we found that the LiPA offers a more-sensitive detection method for HPV genotypes in paraffin-embedded biopsy specimens archived for up to 10 years.

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