Chapter 6
HPV Detection and Genotyping Using the Luminex xMAP Technology

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ABSTRACT
The Human Papillomavirus (HPV) is playing an important role in oral cancer. The molecular detection of HPV is based on the fact that the viral DNA is present in all the epithelial layers of the affected tissue and it can be detected easily with PCR or Real-Time PCR. The major disadvantage of PCR is that it cannot provide genotype information and the Real-Time PCR can only detect very few types in a multiplex assay. Although the HPV typing assays are capable of typing a relatively large spectrum of HPV genotypes, they cannot be automated or deployed in a high-throughput platform. The bead-based technology (Luminex suspension array technology) provides a rapid and cost-effective method to simultaneously detect different HPV genotypes.

BACKGROUND
Over the past 20 years, high-risk human papilloma-virus (HPV) infection has been evolving as a risk factor for the development of head and neck squamous cell carcinoma (Hennessey et al. 2009). In particular, HPV is strongly associated with the development of oropharyngeal cancer and a small minority of oral cavity cancers. In 2008, 47,500 people were diagnosed with head and neck cancer in the United States, representing approximately 3% of new cancer diagnoses, and 11,260 people died (Jemal et al., 2008).
Cervical cancer is the leading cause of cancer mortality among women in developing countries.
There are estimated to be approximately 500,000 new cases of cancer, leading to about 239,000 deaths each year (World Health Report, 2004). Over 99% of cervical cancer cases are linked to genital infection with human papillomavirus, which is the most common viral infection of the reproductive tract worldwide and infects an estimated 660 million people. While HPV infection resolves spontaneously in the majority of people, it can develop into chronic infection and in some women, cervical cancer (WHO, 2005).

The data from combined research studies have revealed the causative role of certain HPV types in cancer. There is sufficient evidence for carcinogenicity of the anogenital tract for types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 (the “high-risk” types of HPV). Jo S et al. (2009) studied the presence of high-risk HPV-16 in patients with HNSCC, assess the impact of HPV status on treatment response and survival in patients treated with combined therapy and the differences in HIF-1alpha and VEGF expression in HPV-positive and -negative tumors. Some case-control studies also also point to a role of HPVs 26, 68, 73 and 82 in cervical cancer, but they are found relatively rarely. There is possible carcinogenicity for HPV 6 and 11, which although not been associated with cervical cancer, are consistently detected in the rare Buschke-Löwenstein tumours converting into verrucous carcinomas of the vulva. In the skin, some types of HPV Genus are possibly carcinogenic to humans. HPV 5 and 8 are considered as carcinogenic for patients with epidermodysplasia verruciformis (WHO, 2005).

There is a strong need not only to detect the virus, but mostly to identify individual HPV types, in order to investigate the epidemiology and the clinical behaviour of particular types and, also to decide the appropriate therapy. Strategies assessed have included vaccination (initiated at age 12 years or at a later age), cytological screening (varying numbers of screening visits using different screening methods, e.g. direct visual inspection, Pap smear, or HPV testing, at different ages), and combined vaccination and screening strategies. The identification of high-risk HPV types prompted the development of new methods for early cancer screening. Furthermore, HPV genotyping is also valuable for investigation of the clinical behavior and epidemiology of particular types, for the characterization of study populations in HPV vaccination trials and for monitoring the efficacy of HPV vaccines.

The molecular detection of HPV is based on the fact that the viral DNA is present in all the epithelial layers of the affected tissue and it can be detected easily with PCR or Real-Time PCR. The major disadvantage of PCR is that it cannot provide genotype information and the Real-Time PCR can only detect very few types in a multiplex assay. In addition, a number of HPV typing assays have recently been reported, such as solid–phase microarrays (Klaassen et al, 2004; Oh et al, 2004), GP5+/6+ - linked enzyme immunoassay (EIA) (van den Brule et al, 2002), the Roche AMPLICOR HPV test, and the INNO-LiPA HPV assay (Labo Biomedical Products; van Ham et al; 2005). Although these assays are capable of typing a relatively large spectrum of HPV genotypes, they cannot be automated or deployed in a high-throughput platform. On the contrary, the bead-based technology (Luminex suspension array technology) provides a rapid and cost-effective method to simultaneously detect different HPV genotypes.

The Luminex xMAP technology uses 5.6 μ polystyrene microspheres which are internally dyed with two spectrally distinct fluorophores, red and infrared. Using different ratios of these fluorochromes, different microspheres sets – up to 100 - can be created, each with a unique spectral signature determined by its red/infrared mixture. Different microspheres sets may be combined within an assay. Since each microsphere carries a unique signature, the xMAP detection system can identify to which set it belongs. Therefore multiplexing up to 100 tests in a single reaction volume is possible. The surface chemistry of the
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