

HPV DETECTION AND TYPING BY MOLECULAR DIAGNOSTIC MICROARRAY

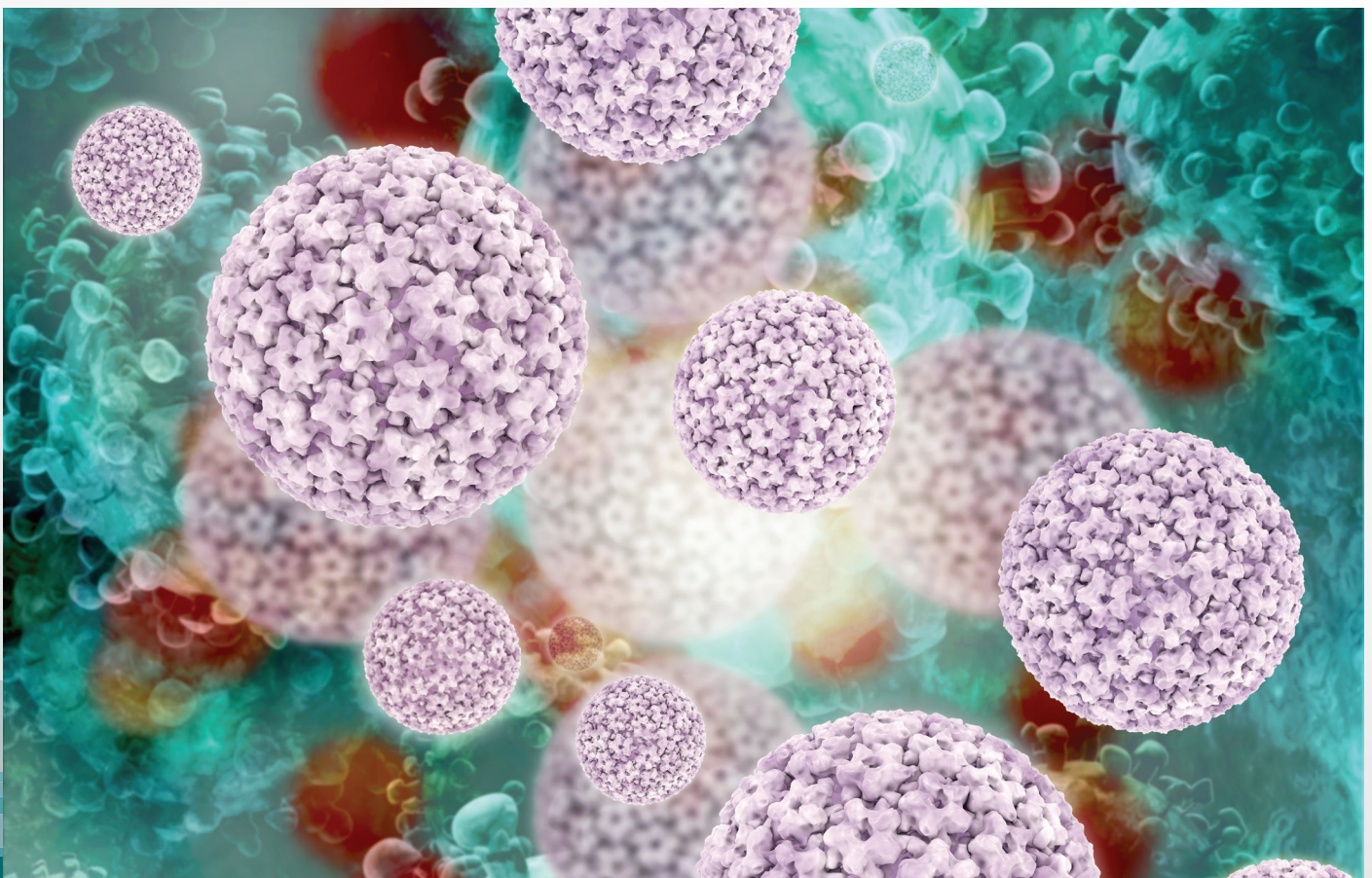
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Human papillomaviruses (HPV) are a major contributory factor to the development of cervical carcinoma. Over recent years it has become evident that effective screening for HPV can significantly reduce the number of cervical cancer cases and deaths, since diagnosis can be made at a very early stage, enabling intervention before the cancer develops. Direct pathogen detection identifies HPV infections even before morphological cell changes have taken place. New developments in multiplex microarray technology have paved the way for highly sensitive HPV tests that allow complete subtyping of all high- and low-risk anogenital HPV. A microarray system with fully automated data analysis is particularly well suited to high-throughput screening.

CERVICAL CARCINOMA

With around 528,000 new cases annually, cervical carcinoma is the fourth most frequent cancer in women worldwide, after breast, colorectal and lung cancers. It is also the fourth most common cause of cancer mortality in women, with 266,000 deaths occurring in 2012 (International Agency for Research on Cancer). In Europe and the USA, cervical carcinoma is the sixth most common malignoma in women.

Cervical carcinoma does not initially cause any pain, and the only symptom may be light bleeding. With increased tumour size, the cancer manifests with a blood-tinged, sweet smelling discharge. Treatment in the early stages involves removal of the altered tissue by conization. In later stages of disease, the uterus and surrounding tissue must be removed.



HPV INFECTION

Infection with genital HPV is a prerequisite for the development of cervical carcinoma. HPV infect epithelial cells, where they are replicated in the cell nuclei. They can cause unregulated tumour-like growth of the host cells, which may be benign, with warts forming at the site of infection, or malignant, as in cervical carcinoma.

However, not all infected women develop cancer: A healthy immune system is able to fight off the infection, and most infected patients eliminate the virus within two years. If the virus remains detectable for longer than 18 months, the infection is considered to be persistent. A persistent infection, in particular with a high-risk HPV subtype, increases the risk of developing cervical carcinoma by around 300-fold.

As the most frequent sexually transmitted viruses, genital HPV have a significant prevalence in the general population. The worldwide prevalence of HPV infection is estimated to be 2-44% in women and 4-45% in men, with regional variations depending on culture and the corresponding sexual activity. Viral transmission from mother to newborn during birth is also possible.

HIGH- AND LOW-RISK SUBTYPES

So far, around 130 HPV subtypes have been described, of which 30 exclusively infect the skin and mucous membranes in the anogenital area. HPV is divided into two groups according to their oncogenic potential. High-risk HPV cause cervical carcinoma. Low-risk HPV alone do not induce tumours, but cause non-malignant tissue changes. Infections with multiple HPV subtypes are common.

Of the high-risk anogenital types, HPV 16 is found in 50-60% of cervical carcinomas and HPV 18 in 10-20%. Other types classified as high-risk by the WHO are 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66. Types 26, 53, 68, 73 and 82 have also been detected in cervical carcinoma and should be considered as high-risk types. For this reason, in cancer risk assessment it is important not only to differentiate between high- and low-risk types, but also to discriminate between the different high-risk viruses.

Of the low-risk types, HPV 6 and 11 are the main causative agents of genital warts (condylomata acuminata, fig warts). Further low-risk types are 40, 42, 43, 44, 54, 61, 70, 72, 81 and 89. Although not lethal, low-risk HPV cause discomfort and embarrassment for patients. Low-risk HPV can also cause mild dysplasia. In these cases HPV subtyping is useful for excluding a high-risk HPV infection and a corresponding risk of cervical cancer.

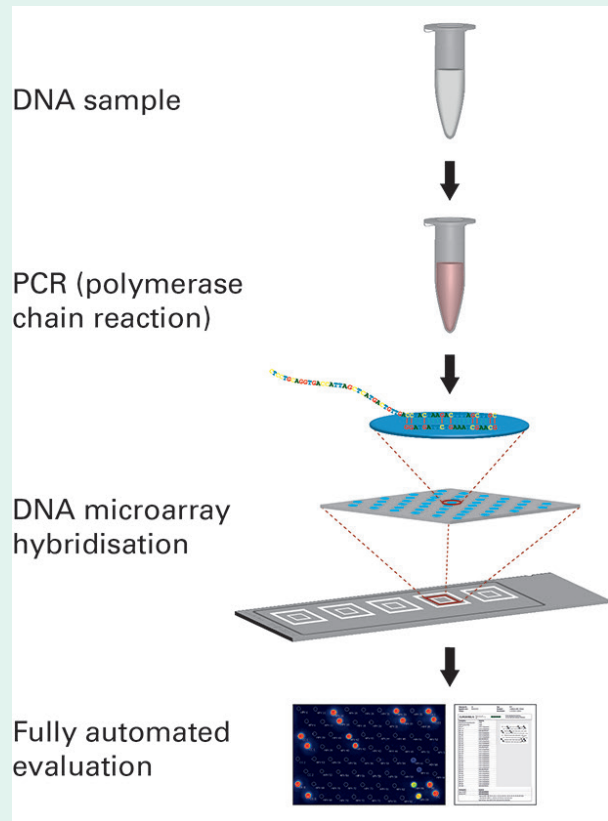
HPV DIAGNOSTICS

Along with the current gold standard Papanicolaou (Pap) test, HPV direct detection plays an important role in the early diagnosis of cervical carcinoma. In contrast to the Pap test, which is used to investigate cervical cells for pathological changes, PCR-based methods detect viral nucleic acids directly, and can thus identify an HPV infection at a very early stage before morphological cell changes have even occurred. Moreover, while the Pap test is based on subjective evaluation, HPV detection represents an objective as well as extremely sensitive test method.

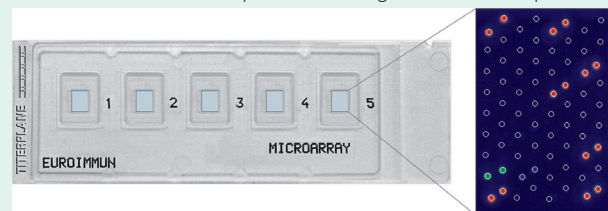
A positive result for high-risk HPV indicates an increased risk for cervical carcinoma, which can then be minimised by more frequent follow-up examinations to detect morphological cell changes at an early stage. Based on the recommendations of the respective professional societies, HPV-negative women can forgo Pap smears for a longer time interval.

The PCR detection strategy is a critical aspect of direct HPV analysis. Tests based on conserved genes like L1 may yield false negative results in some cases due to loss of these genes during integration of the viral DNA into the host DNA. The highest possible detection sensitivity is achieved using the viral oncogenes E6/E7. Detection of variable sequences in these genes enables differentiation of the different HPV subtypes. ➔

▼ FIGURE 1: EUROArray procedure



▼ FIGURE 2: EUROArray slide and enlargement of a biochip



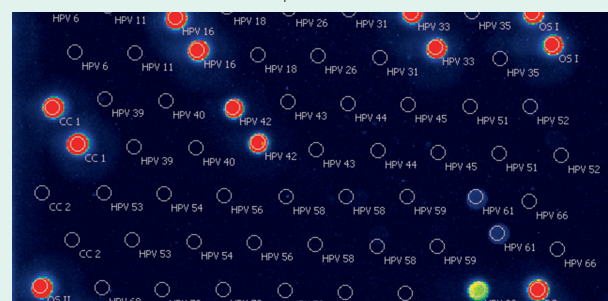
▼ FIGURE 3: Microarray evaluation by EUROArrayScan

Patient ID : 26	Test : HPV
Result from : 08.08.2013	Protocol : 130808LH_HP
Lot :	Print date : 14.02.2014 13:22:06

EUROIMMUN	Medizinische Laboragnostika AG		Automatic evaluation with the EUROArrayScan software
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Partial result	Result
Cross contamination control	valid
DNA positive control	valid
HPV 6**	not detected
HPV 11**	not detected
HPV 16*	detected
HPV 18*	not detected
HPV 26*	not detected
HPV 31*	not detected
HPV 33*	detected
HPV 35*	not detected
HPV 39*	not detected
HPV 40**	not detected
HPV 42**	detected
HPV 43**	not detected
HPV 44**	not detected

▼ FIGURE 4: Patient result report



IN THE KNOW

COMPLETE HPV TYPING

A standardised microarray based on PCR detection of E6/E7 has been developed for complete HPV typing in routine diagnostics. Using an extensive panel of specific primers and probes, the EUROArray HPV detects all thirty genitally relevant HPV subtypes in one test, distinguishing 18 high-risk subtypes that may trigger cancer (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) and 12 low-risk subtypes that cause benign warts (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89). Multiple infections are reliably identified, and primary and persistent infections can be differentiated.

SIMPLE PROCEDURE WITH AUTOMATED EVALUATION

The EUROArray procedure (see figure 1) is extremely easy to perform, requiring only limited expertise in molecular biology. DNA prepared from patient cervical smear samples is first amplified by multiplex polymerase chain reaction (PCR). The fluorescently labelled PCR products are then incubated with biochip microarray slides (see figure 2) containing immobilised complementary DNA probes. Specific binding (hybridisation) of the PCR products to their corresponding microarray spots is detected using a specialised microarray scanner.

In contrast to manually evaluated tests, the results are evaluated (see figure 3) and interpreted fully automatically by user-friendly software (EUROArrayScan). A detailed result report (see figure 4) is produced for each patient and all data is documented and archived.

Meticulously designed primers, ready-to-use PCR components and integrated controls all contribute to the reliability of the analysis. The entire EUROArray process from sample arrival to report release is IVD validated and CE registered, supporting quality management in diagnostic laboratories.

CLINICAL EVALUATION

In a clinical study, 188 DNA samples that had been precharacterised using other HPV test systems were analysed using the EUROArray HPV microarray. In all cases the determination was successful, and results were identical or in agreement with the precharacterisation, taking into account the different test specifications.

PERSPECTIVES

HPV testing is a powerful cancer prevention tool, providing crucial support to Pap screening programmes. Proponents of HPV screening advocate that it could even eventually replace the Pap test as the first-line screening method for cervical cancer. The International Agency for Research on Cancer states there is sufficient evidence that testing for HPV infection as the primary screening modality can reduce cervical cancer incidence and mortality rates. The state-of-the-art EUROArray test system is ideally suited to the high-throughput requirements of routine screening, providing fast and sensitive detection of all relevant high- and low-risk HPV subtypes, combined with fully automated data analysis. [\[4\]](#)

