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SCREENING
TRENDS IN DRUG DISCOVERY
inside

GIT VERLAG
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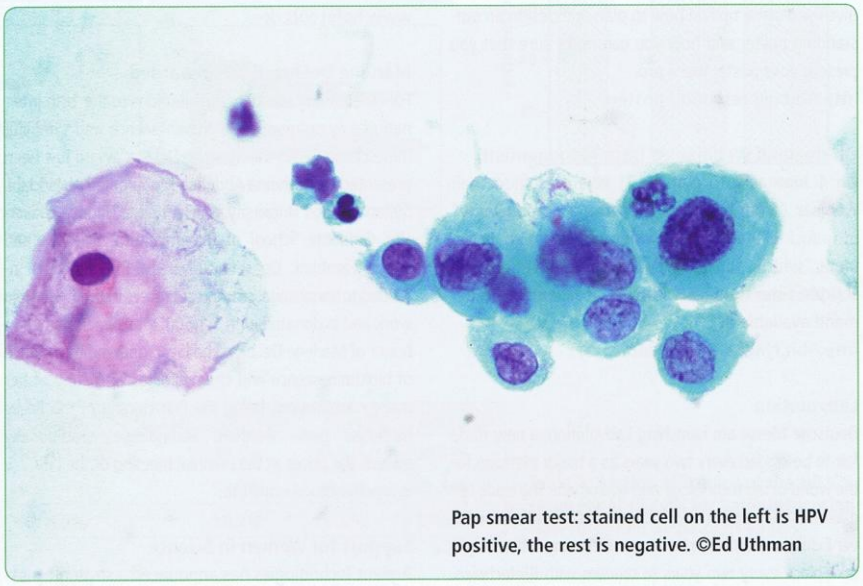
Automatic Array Hybridization

Characterization of Human Papillomaviruses by PCR-Screening

Cervical cancer is the second most common cancer in women worldwide. High-risk strains of human papillomaviruses can be detected in nearly 100 % of diseased tissue. Altogether, more than 124 types of this widespread, mainly sexually transmitted virus are described. The cytological routine diagnostic methods used until now are labor intensive and results are highly depending on skills and expert knowledge of the qualified user.



Pap smear test: stained cell on the left is HPV positive, the rest is negative. ©Ed Uthman

Alternatively, PCR-screening combined with DNA probe hybridization has been established as highly sensitive method. However, for high-throughput screenings, the commonly used line probe technology quickly reaches its limits. To overcome this, a DNA Array in 96-well microtiterplate format was developed. This array format provides professional users a simple, efficient and automatable HPV diagnostic and strain typing along with high throughput screening.

The HPV array combines the established, robust and sensitive PCR with subsequent reverse hybridization and the simultaneous analysis of 96 samples in one plate. Easy increase of the sample amount faces only insignificant additional expense compared to the line probe technology. The 96-well plate format is generally established as standard in automated systems.

Cytology

The 'Pap-Smear' (named after originator Mr. Papanicolaou) is a gold standard method for cervix cancer screening: Epidermal cells contained in a cervical scrape are placed on a microscope slide, fixed and stained. The stained cells are then analysed by microscope for abnormal size and structure. Results are classified according to international standards. Training and experience is necessary for exact diagnosis of different stages of cellular abnormalities.

HPV-PCR screening will not substitute Pap-Smear, but can reduce it only to these women, with detected high risk types of HPV.

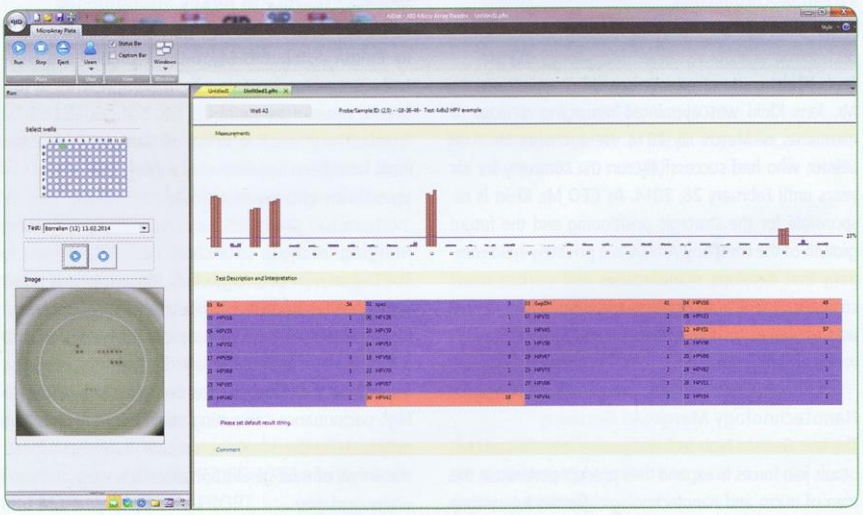
HPV Screening by Multiplex-PCR with Reverse Hybridization

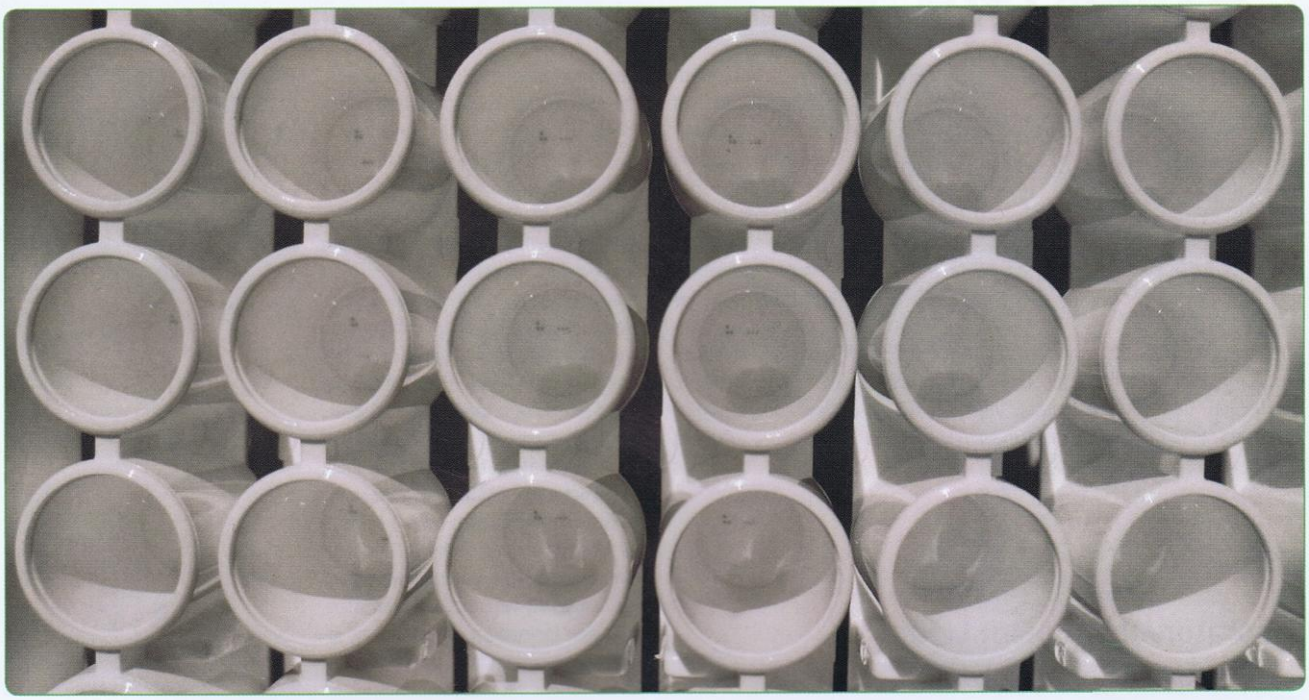
The assay starts with total DNA from biopsy material, isolated by column or magnetic bead extraction methods. A qualitative multiplex PCR with several primer pairs (partly degenerated) amplifies a 150 bp fragment of the E1 gene of all detectable HPV subtypes as well as a GAPDH fragment as internal control. By using biotinylated primers the PCR product is labeled and can be used without further purification for hybridization with the target sequence for HPV type identification. PCR and purification needs at least 2.5 h.

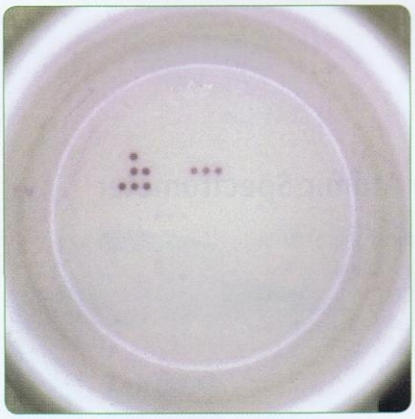
The E1 gene codes for HPV regulatory early protein 1 which is involved in the initiation of viral genome replication and organization of the 'replisome'. It neither has a special role in cancer genesis nor is it a biomarker therefore.

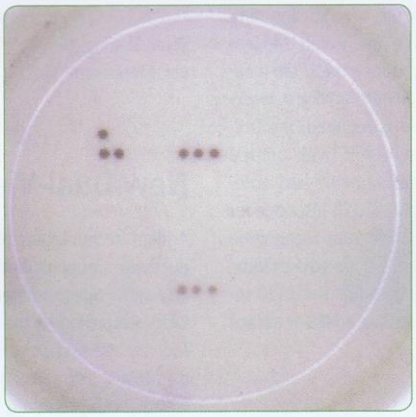
For reverse hybridization the labeled PCR-products are denatured into single strand fragments and applied to immobilized DNA-Probes with specific sequences for the different HPV types. During hybridization at 47°C the biotinylated single strands bind to their targets. A stringent wash step avoids unspecific binding.

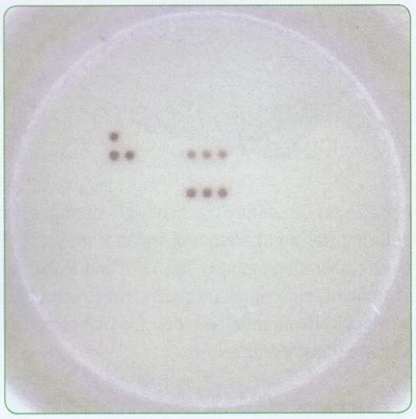
A streptavidin-conjugated alkaline phosphatase reaction shows amplicon and probe hybrids. By adding NBT/BCIP those hybrids are vi-











sualized as a violet colored precipitate and can be interpreted manually or by a reader system. This hybridization process takes about 1,5 – 2 h.

In Line Blot assays the probes are immobilized on nitrocellulose strips and the assay is performed manually or in autoblotting devices with up to 40 strips. The probe number is limited by the length of the strips. The handling of the strips makes it difficult to process large sample numbers.

The HPV Array described here, brings the hybridization from the strip into the well of a 96 well microtiter plate. Here the DNA probes are applied in a defined spot pattern onto the bottom of the wells. By using a microarrayer for applying the probes much more different DNA probes can be located on the test area compared to the NC-strip. The hybridization process takes place in the well. The assay can be processed on ELISA pipetting workstations with 96 samples a plate. We offer a suited device in cooperation with Titertek-Berthold, Pforzheim.

The array enables the simultaneous detection of six low- and 23 high-risk types including clinically relevant high-risk types according to the WHO classification system. In particular, this includes HPV 16, 18 and 45, which are detectable in 70% (HPV 16, 18), respectively 7% (HPV 45), of all cervical carcinomas.

To ensure correct assay performance, different control probes are included:

(i) a conjugation control, (ii) an amplification control (GAPDH) for appropriate DNA extraction, PCR and hybridization and (iii) a specification control to eliminate the risk of false-positive results when hybridization conditions did not meet the manufacturer's recommendations, e.g. when washing temperature was too low.

Automatic Analysis of 29 HPV-Genotypes

As the probes are loaded on the wells in triplicates, one stained triplet corresponds to one HPV subtype and can easily be identified by its position.

Due to the small size of the spots in this array system, the evaluation is hardly possible using the naked eye. Therefore, we offer a reader system for array analysis. Integration of verified and approved results, in existing Lab-information or - management software is easily possible.

A substantial advantage is the significant larger sample number per experiment compared to the line probe assay, reducing the workload significantly. In addition, the kit is designed for time- and labor-saving automated hybridization. Another advantage is the small assay format minimizing the amount of chemicals, the waste volume and necessary work space. In conclusion, the HPV-DNA array facilitates an efficient HPV-screening as an initial step before cytological diagnostic procedures and can therefore be a crucial component of HPV prevention.

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