AUTOIMMUN DIAGNOSTIKA GMBH

TITERTEK BERTHOLD

Reverse Hybridisation of DNA probe array with Crocodile miniWorkstation

Bettina Plommer [1] Alfons Weilbach [2]

[1] Berthold Detection Systems GmbH (Titertek-Berthold), Pforzheim, Germany, plommer@titertek-berthold.com [2] AID GmbH, Strassberg, Germany, weilbach@aid-diagnostika.com

Introduction

DNA arrays enable to detect a variety of specific targets in one single experiment, even with small sample material. Here we describe the characterisation of Human Papilloma Virus (HPV) types by reverse hybridisation on a DNA probe array on the bottom of a 96 well plate. Hybridisation requires various dispense, incubation and wash steps at different temperatures. Thus automation is highly recommended.

Material & Methods

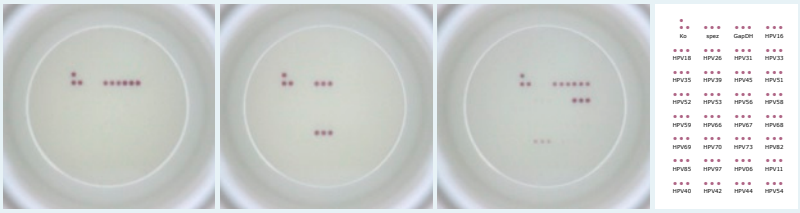
- HPV DNA Array (AID GmbH)
- HPV samples
- Crocodile miniWorkstation (Titertek-Berthold)
- ELISpot reader (AID GmbH)

After PCR HPV samples were transferred to the wells of the HPV DNA Array plate. Complete reverse hybridisation was processed automatically by using the Crocodile miniWorkstation. *Table 1, Picture 3* Signal analyzing was done by using ELISpot Reader with AIDot software. *Picture 4, Picture 2*

Results

Various HPV types could be detected as each HPV type shows a different pattern.

Picture 5
Signal of HPV16-1, HPV06, HPV16 42 57 according to HPV type pattern



Conclusion

The HPV DNA Array is an easy and fast method to characterize HPV types by reverse hybridisation. Various different HPV types can be detected using only one well of a 96 well plate. Crocodile miniWorkstation provides a convenient method for automation of reverse hybridisation. Total assay time for the automated assay is approximately the same as when the assay is performed manually but operator hands-on time is reduced from about 3 h to 20 minutes.

Picture 1
HPV DNA Array with HPV specific probes printed on the bottom of the wells

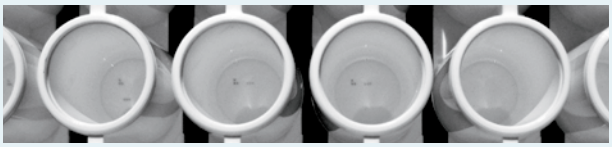
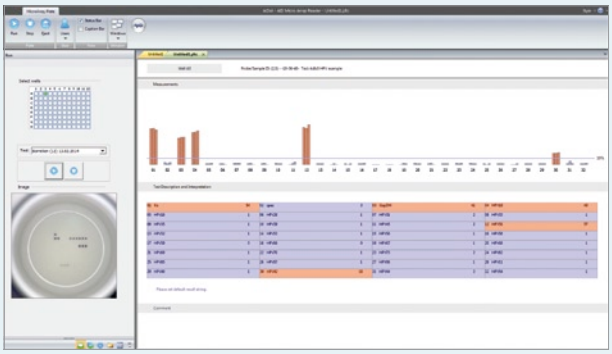


Table 1
Protocol of reverse hybridisation (shortform)

Step	Temp / Volume / Time
Dispense Hybrid	200 µl
Incubate	30 min at 47°C
Stringent Wash	5x
Shake	30 sec
Dispense Conjugate	200 µl
Incubate	30 min at RT
Wash	3x
Shake	30 sec
Dispense Substrate	200 µl
Incubation	10 min at RT
Wash	3x

Picture 2
AIDot software



Picture 3
Crocodile miniWorkstation

Picture 4
ELISpot reader

Reverse Hybridisation of DNA probe array I60417