VisionArray
HPV High Risk Chip 1.0

REF VA-0002-10 Σ 10 tests

REF VA-0002-50 Σ 50 tests

For the specific detection of 24 Human Papilloma Virus (HPV) Types that have been produced with the help of the VisionArray HPV Primer Kit 2.0.

CE IVD

In vitro diagnostic medical device
according to EU directive 98/79/EC

1. Intended use

The VisionArray HPV High Risk Chip 1.0 is intended to be used with the VisionArray Analysis Package for the qualitative detection and genotyping of PCR-amplificates of 24 clinically relevant Human Papilloma Virus (HPV) High Risk and probably High Risk genotypes that have been produced with the help of the VisionArray HPV Primer Kit 2.0 or the VisionArray HPV PreCise Master Mix.

This product is designed for in vitro diagnostic use (according to EU directive 98/79/EC). Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data of the patient by a qualified pathologist.

2. Clinical relevance

Infections with HPV are common and a major risk factor for the development of e.g. cervical carcinoma. At present, there are more than 150 different HPV types described. Depending on their risk to induce cancer, they are divided into Low Risk (LR), probably High Risk and High Risk (HR) types.

The VisionArray HPV High Risk Chip 1.0 is designed to detect the following 24 High Risk and probably High Risk genotypes:

Classification of the 24 HPV genotypes on the VisionArray HPV High Risk Chip 1.0

High Risk	Probably High Risk
16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	26, 34, 53, 66, 67, 68a, 68b, 69, 70, 73, 82IS39, 82MM4

The HPV-Types were classified according to the current scientific literature.

3. Test principle

DNA-fragments with a specific sequence are detected from a pool of DNA-fragments on a glass chip with the help of immobilized DNA capture sequences by DNA/DNA-hybridization. For this detection system DNA-samples from formalin-fixed, paraffin-embedded tissue or cell samples can be used as raw material. As a first step, the target sequences in these samples have to be amplified and biotinylated in a PCR. The hybridization between the amplified sequences and the complementary DNA captures is performed subsequently. After the hybridization, the unspecifically bound DNA is washed away by short stringent wash steps. The specific bound biotinylated sequences are secondary labeled with a Streptavidin-Peroxidase-Conjugate afterwards and visualized by a tetramethylbenzidine (TMB) staining.

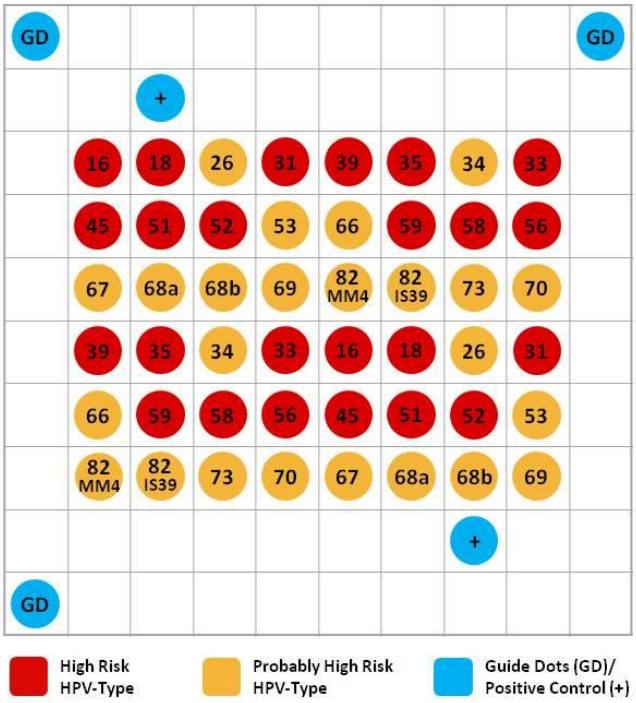
4. Reagents provided

The following components are included:

Code	Components	Quantity 10	Quantity Σ 50
VA-0002	VisionArray HPV High Risk Chip 1.0	10	5x10
	Instructions for use	1	1

Description of the Chip:

Positioning of the capture sequences on the chip:



5. Materials required but not provided

- VisionArray Analysis Package (E-4060)
- VisionArray HPV Primer Kit 2.0 (VP-0001) or VisionArray HPV PreCise Master Mix (ES-0007)
- VisionArray Detection Kit (VK-0003)

The VisionArray Analysis Package has to contain the VisionArray HPV High Risk Chip File 1.0 (E-4202) for a successful scan.

6. Storage and handling

The chips have to be stored in the intact original packing at -16…-22°C. If these storage conditions are followed, the chips are stable, without loss of performance, at least until the expiry date printed on the label.

After opening the original packaging store at -16…-22°C and use the chips within two months.

7. Warnings and precautions

- Read the instruction for use prior to use!
- Do not use the chips after the expiry date has been reached!
- Chips should be used in a dust-free setting. Avoid the contamination of the chip surface with dust or other particles!
- Avoid direct contact with the array field on the chip-surface!
- Only the labeled side of the slide can be used for hybridization.
- Avoid cross-contamination of samples as this may lead to erroneous results.

8. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data by a qualified pathologist.
- The chip should be used only for detecting the HPV types described in 2. "Clinical relevance".

Furthermore, following factors can influence the detection system:

- Deviation from the proposed detection protocol (e.g. temperature or volumes of the reagents).
- Degraded or low concentrated DNA material.
- Inappropriate raw material.
- Use of not calibrated or impaired equipment.
- In strong HPV infections or in case of multiple infections the intensity of the positive control might be impaired.
- Do not work under laminar flow during the assay procedure since this might lead to an impairment of the results.

9. Interfering substances

- Low PCR efficiency due to PCR inhibitors in DNA raw material (e.g. blood).
- Use of PCR additives that could influence the hybridization (e.g. DMSO, betaine, urea).

10. Preparatory treatment of specimens

Starting material for this detection system are PCR amplification products that have been produced with the Vision*Array* HPV Primer Kit 2.0 or the Vision*Array* HPV PreCise Master Mix.

The hybridization and detection of the chips has to be performed with the Vision*Array* Detection Kit according to the instructions for use.

11. Preparatory treatment of the device

Bring chips to room temperature (18…25°C) before use.

12. Assay procedure

Perform the scan according to the instruction for use of the Vision*Array* Analysis package.

13. Interpretation of results

With the aid of the Vision*Array* HPV High Risk Chip 1.0, it is possible to make a qualitative statement about the presence or absence of one or more of 24 HPV-types in the investigated sample.

The intensity of the signals is influenced by the prevalence of the target sequences in the sample as well as miscellaneous factors of the detection system. The absolute numbers of the signal intensity cannot be used for quantification of the DNA concentration.

Software-Based Evaluation

The automated evaluation of the results is performed by the Vision*Array* Analyzer Software. A comprehensive manual for a chip-analysis is enclosed to the Software.

14. Recommended quality control procedures

Internal controls:

- Guide dots/Hybridization control (GD): These dots are used by the Vision*Array* Analyzer Software for the positioning of the grid. Additionally, staining of the guide dots is proof for a successful hybridization, labeling, and staining reaction and is used for the calculation of the relative intensity of the signals.
- Positive control/PCR-control (+): These controls are used for the evaluation of the PCR-reaction and the quality of the PCR-template.
- All capture sequences and the positive control are set up on the chip as duplicates and the guide dots as triplicates. The signals are visible on the chip as circular hybridization signals.

If any of these controls fail to show complete staining, the results are not valid.

External controls:

In order to monitor correct performance of processed specimens and test reagents, each assay should be accompanied by external validated positive and negative control specimens. If internal and/or external controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

15. Performance characteristics

15.1 Analytical performance:

For analytical specificity and sensitivity of the Vision*Array* HPV High Risk Chip 1.0 each of the 24 HPV catcher sequences were tested separately together with 17 low risk HPV types. Therefore, sequence verified plasmids with a concentration of 50-500,000 genome equivalents (GEQ) were tested.

Specificity and limit of detection for all 24 HPV-Types

HPV Type	Specificity [%]	Limit of Detection (GEQ)
16 (HR)	100	50
18 (HR)	100	50
26	100	500
31 (HR)	100	500
33 (HR)	100	50
34	100	50
35 (HR)	100	50
39 (HR)	100	50
45 (HR)	100	50
51 (HR)	100	50
52 (HR)	100	500
53	100	500
56 (HR)	100	50
58 (HR)	100	500
59 (HR)	100	5,000
66	100	500
67	100	50
68a	100	500,000
68b	97.6	500
69	100	500
70	100	50
73	100	5,000
82IS39	100	50
82MM4	100	500

The sensitivity of the analysis system was tested for each HPV type separately. The sensitivity depends on the amount and efficiency of the PCR-cycles and the affinity of the catchers.

The determined sensitivity refers to the detection of a single target sequence. The detection of a multiple infection can lead to impairment of the sensitivity of some HPV-types, due to competition during the PCR-reaction, especially in mixed samples with a strong difference in the concentration.

The performance was validated using the procedures described in this instruction for use. Modifications to these procedures might alter the performance and have to be validated by the user.

15.2 Cross hybridizations:

- When present in high concentrations, HPV 68a hybridized to HPV 68b in 100% of the cases. In lower concentrations no cross hybridization could be observed. However, HPV 68b is a subtype and therefore in high concentrations not distinguishable from HPV 68a.

15.3 Cutoff

For the evaluation of the results the dot size is set to 50.

The threshold (cutoff) was set to 25 for the greyscale image of this dot size. A signal below this value is considered background by the Vision*Array* Analyzer Software.

16. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

17. Troubleshooting

Any deviation from the operating instructions can lead to impairment of the detection reaction of the target sequence.

Problem	Possible cause	Action
No signal	Wrong temperature	Check the hybridization temperature
	Expired reagents	Check the reagents
Only guide dots and no other signals	Problems with the PCR-product (PCR not efficient enough or DN- template degraded)	Check PCR efficiency with a positive control; Check PCR-chemicals and thermal cycler program; Check PCR-product in agarose gel
	Wrong raw material	Check the raw materials
	Wrong combination of chip and sample	Check the sample/chip combination
Only guide dots and PCR-control, but no other signals	No target sequence present	Use positive control
Only guide dots and HPV Signals, but no positive control	Strong HPV infection or multiple HPV infection	Dilute sample DNA
	Degraded sample	New DNA extraction; store at -16…-22°C
Too much background	Incubation time of Detection Solution or Blue Spot Solution too long; temperature during incubation too high	Check incubation time and temperature of Detection Solution and Blue Spot Solution
	Slides not properly dried	Check drying step
Strong, leaking signals	Incubation time of Detection Solution or Blue Spot Solution too long or temperature too high	Stepwise adjustment of the incubation time and temperature of Detection Solution and Blue Spot Solution
Weak signals	Hybridization temperature incorrect	Check temperature
	Hybridization time too short	Extend hybridization time to a maximum of 30 min
	Incubation time of Detection Solution or Blue Spot Solution too short	Extend incubation time of Detection Solution and Blue Spot Solution
	Weak PCR amplification/ bad quality of the DNA-template	Check DNA-template
Cross-hybridization signals, false positive signals	Contamination of the PCR-chemicals or PCR-product	Replace the PCR-chemicals in use
	Contamination during the preparation of the PCR or of the hybridization mix	Avoid transfer of sample during the preparation of the mix
	Hybridization temperature too low	Check hybridization temperature
	Several chips incubated too long in the same wash buffer	Swift execution of the washing steps
Single signal instead of duplicates	Mechanical elimination of the second signal, e.g. due to contact with the pipette tip	Avoid direct contact with the array field
	Irregular covering of the array field due to air bubbles	Apply solutions without air bubbles
	Weak signals around the threshold (1 above and 1 below)	Repeat PCR and detection under consideration of the conditions required in the manual

18. Literature

- IARC Monographs on the evaluation of carcinogenic risks to humans, Vol. 100, 2012; ISBN 978 92 832 1319 2
- WHO Human Papillomavirus Laboratory Manual, First edition, 2009.
- Schmitt M, et al. (2008) Journal of Clinical Microbiology 46:1050-1059.
- Schmitt M, et al. (2013) International Journal of Cancer 132:2395-2403.

Our experts are available to answer your questions.
Please contact helptech@zytovision.com

42 life sciences GmbH & Co. KG
Fischkai 1
27572 Bremerhaven/Germany
Phone: +49 471 4832-500
Fax: +49 471 4832-308
www.42ls.com
Email: info@42ls.com

Distributed by:
ZytoVision GmbH
Fischkai 1
27572 Bremerhaven/ Germany
Phone: +49 471 4832-300
Fax: +49 471 4832-509
www.zytovision.com
Email: info@zytovision.com