# Vision*Array*

# HPV Chip 1.0

REF VA-0001-10 Σ 10 tests

REF VA-0001-50 Σ 50 tests

For the specific detection of 41 Human Papilloma Virus (HPV)-types that have been produced with the help of the Vision*Array* HPV Primer Kit.

CE

IVD

In vitro diagnostic medical device

according to EU directive 98/79/EC

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**Manufactured by:**

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

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# Contents

# 1 Product Description

## 1.1 Scope of Application

The Vision*Array* HPV Chip 1.0 is intended to be used with the Vision*Array* Analysis Package for the qualitative detection and genotyping of PCR amplificates of 41 clinically relevant Human Papilloma Virus (HPV) genotypes that have been produced with the help of the Vision*Array* HPV Primer Kit and the Vision*Array* Detection Kit.

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

The Vision*Array* HPV Chip 1.0 is designed to detect the following 41 genotypes:

**Risk classification of the 41 HPV genotypes on the Vision*Array* HPV Chip 1.0**

| **High Risk** | **Probably High Risk** | **Low Risk** |
|---|---|---|
| 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 | 26, 34, 53, 66, 67, 68a, 68b, 69, 70, 73, 82 (IS39), 82 (MM4) | 6, 11, 40, 42, 43, 44, 54, 55, 57, 61, 62, 72, 81 (CP8304), 83 (MM7), 84 (MM8), 90, 91 |

The HPV types were classified according to the current scientific literature (refer section 6 Literature).

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.

## 1.2 Product

VA-0001-10: 10 tests

VA-0001-50: 50 tests

## 2 Basic Principles

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.

## 3 Safety Precautions and Disposal

- ✓ Read the operation instructions 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 labelled side of the slide can be used for hybridization.

# 4 The Vision*Array* HPV Chip 1.0

## 4.1 Components

The following components are included:

| Code | Components | Quantity | |
|---|---|---|---|
| | | **10** Σ | **50** |
| VA-0001 | Vision*Array* HPV Chip 1.0 | 10 | 50 |
| | Instruction manual | 1 | 1 |

## 4.2 Storage and Shelf Life

The chips have to be stored in the intact original packing at room temperature. 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 use the chips within one month.

## 4.3 Test Material

Starting material for this detection system are PCR amplification products that have been produced with the Vision*Array* HPV Primer Kit (Article-No. VP-0001-50).

The hybridization and detection of the chips has to be performed with the Vision*Array* Detection Kit (Article-No. VK-0003-50) according to the specifications of the manufacturer.

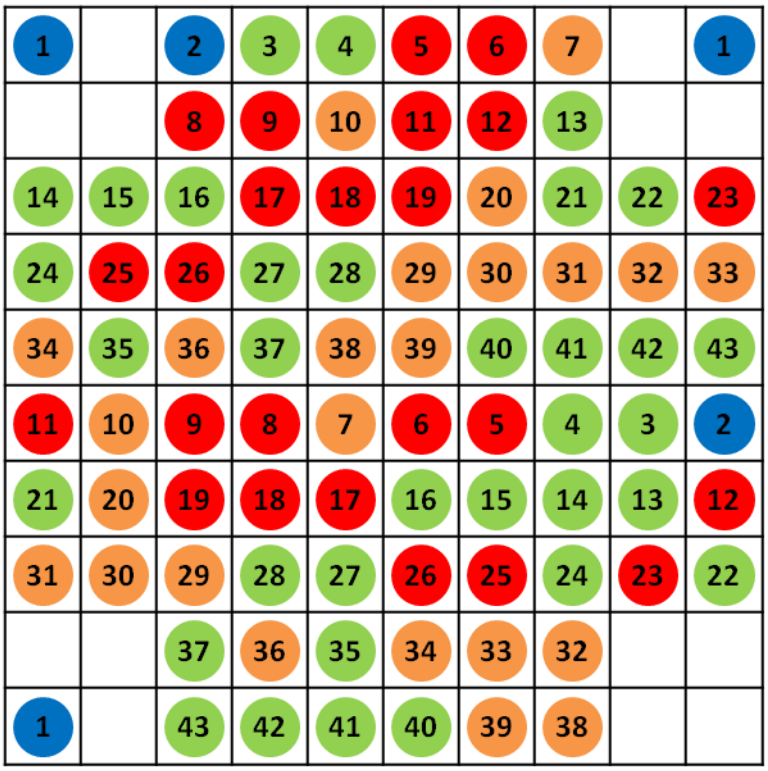
## 4.4 Additional Material

The chips have to be analyzed on the Vision*Array* Analysis Package (Article-No. E-4060-1).

The Vision*Array* Analysis Package has to contain the Vision*Array* HPV Chip File 1.0 for a successful scan.

## 4.5 Description of the Chip

Positioning of the capture sequences on the chip:




Allocation position and capture sequence:

| No. | Capture sequence | No. | Capture sequence | No. | Capture sequence | No. | Capture sequence |
|---|---|---|---|---|---|---|---|
| 1 | Guide Dots | 12 | HPV 39 | 23 | HPV 56 | 34 | HPV 70 |
| 2 | Positive control | 13 | HPV 40 | 24 | HPV 57 | 35 | HPV 72 |
| 3 | HPV 6 | 14 | HPV 42 | 25 | HPV 58 | 36 | HPV 73 |
| 4 | HPV 11 | 15 | HPV 43 | 26 | HPV 59 | 37 | HPV 81 (CP8304) |
| 5 | HPV 16 | 16 | HPV 44 | 27 | HPV 61 | 38 | HPV 82 (IS39) |
| 6 | HPV 18 | 17 | HPV 45 | 28 | HPV 62 | 39 | HPV 82 (MM4) |
| 7 | HPV 26 | 18 | HPV 51 | 29 | HPV 66 | 40 | HPV 83 (MM7) |
| 8 | HPV 31 | 19 | HPV 52 | 30 | HPV 67 | 41 | HPV 84 (MM8) |
| 9 | HPV 33 | 20 | HPV 53 | 31 | HPV 68a | 42 | HPV 90 |
| 10 | HPV 34 | 21 | HPV 54 | 32 | HPV 68b | 43 | HPV 91 |
| 11 | HPV 35 | 22 | HPV 55* | 33 | HPV 69 | | |

*HPV 55 is classified by now as subtype of HPV44, but is still labelled HPV55 for consistency reasons.

High Risk HPV
Guide Dot, positive control
probably High Risk HPV
Low Risk HPV

In addition to the 41 HPV genotypes following controls are situated on the chip:

- Guide dots (Hybridization control): 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.

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

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.

# 5 Interpretation of Results

## 5.1 General

With the aid of the Vision*Array* HPV Chip 1.0, it is possible to make a qualitative statement about the presence or absence of one ore more of 41 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.

## 5.2 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.

## 5.3 Specificity of the Tests

### 5.3.1 Analytical Specificity

Analytical specificity of the Vision*Array* HPV Chip 1.0 was tested for each of the 41 HPV types separately. Therefore, sequence verified plasmids with a concentration of ≥ 100,000 genome equivalents (GEQ) of the 40 other HPV types were tested in triplicates. For this purpose, all HPV types were amplified by PCR, hybridized on the chip and subsequently detected.

Specificity is calculated as followed:
TN/( TN + FP) [*TN=True negative signals; FP= False positive signals]*

**Specificity for all 41 HPV-types**

| HPV Type | Specificity [%] |
|---|---|
| 6 | 100 |
| 11 | 100 |
| 16 (HR) | 100 |
| 18 (HR) | 100 |
| 26 | 100 |
| 31 (HR) | 100 |
| 33 (HR) | 100 |
| 34 | 100 |
| 35 (HR) | 100 |
| 39 (HR) | 100 |
| 40 | 99.2 |
| 42 | 100 |
| 43 | 100 |
| 44 | 100 |
| 45 (HR) | 100 |
| 51 (HR) | 100 |
| 52 (HR) | 100 |
| 53 | 100 |
| 54 | 100 |
| 55 | 100 |
| 56 (HR) | 100 |
| 57 | 100 |
| 58 (HR) | 100 |
| 59 (HR) | 100 |
| 61 | 100 |
| 62 | 100 |
| 66 | 100 |
| 67 | 100 |
| 68a | 100 |
| 68b | 97.6 |
| 69 | 100 |
| 70 | 100 |
| 72 | 100 |
| 73 | 100 |
| 81 (CP8304) | 98.4 |
| 82 (IS39) | 100 |
| 82 (MM4) | 100 |
| 83 (MM7) | 100 |
| 84 (MM8) | 100 |
| 90 | 100 |
| 91 | 100 |

### 5.3.2 Cross hybridizations:

- In 33% of the cases HPV 70 hybridized to HPV 40. However, in lower concentrations no cross hybridization could be observed.
- In 66% of the cases HPV 62 hybridized to HPV 81. However, in lower concentrations no cross hybridization could be observed.
- In 100% of the cases HPV 68a hybridized to HPV 68b. In lower concentrations no cross hybridization could be observed. However, HPV 68b is a subtype and therefore in high due to the cross hybridisations not distinguishable from HPV 68a.

## 5.4 Sensitivity of the test

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.

In order to estimate the sensitivity, cloned DNA-samples of the HPV types were tested in 3-6 different dilutions ranging from 50 GEQ to 500,000 GEQ. The lowest concentration that could be detected in at least 66 % of the cases was set as the limit of detection (LOD) of the analyte. Only for HPV 16 and 18 the WHO Human Papillomavirus Laboratory Manual (First edition, 2009) suggests a LOD of 50 GEQ.

**Limit of Detection for all 41 HPV-types**

| HPV Type | Limit of Detection (GEQ) |
|---|---|
| 6 | 50 |
| 11 | 50 |
| 16 (HR) | 50 |
| 18 (HR) | 50 |
| 26 | 500 |
| 31 (HR) | 5,000 |
| 33 (HR) | 50 |
| 34 | 50 |
| 35 (HR) | 50 |
| 39 (HR) | 50 |
| 40 | 50 |
| 42 | 500 |
| 43 | 500 |
| 44 | 500 |
| 45 (HR) | 50 |
| 51 (HR) | 50 |
| 52 (HR) | 500 |
| 53 | 500 |
| 54 | 50 |
| 55 | 50,000 |
| 56 (HR) | 50 |
| 57 | 500 |
| 58 (HR) | 500 |
| 59 (HR) | 5,000 |
| 61 | 500 |
| 62 | 500 |
| 66 | 500 |
| 67 | 50 |
| 68a | 500,000 |
| 68b | 500 |
| 69 | 500 |
| 70 | 50 |
| 72 | 5,000 |
| 73 | 5,000 |
| 81 (CP8304) | 50 |
| 82 (IS39) | 50 |
| 82 (MM4) | 500 |
| 83 (MM7) | 500 |
| 84 (MM8) | 5,000 |
| 90 | 500 |
| 91 | 500 |

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 probes with a strong difference in the concentration. In extremely high concentrated HPV-probes the intensity of the positive control might be impaired as well.

### 5.5 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 Vison*Array* Analyzer software.

### 5.6 Limits of the Method

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.

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

## 6 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.

# 7 Problems and Possible Causes

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 raw material | Check the raw materials |
| | Wrong combination of chip and sample | Check the sample/chip combination |
| | 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 DNA template degraded) | Check PCR efficiency with a positive control;<br>Check PCR chemicals and thermal cycler program;<br>Check PCR product in agarose gel |
| Only guide dots and PCR control, but no other signals | No target sequence present | Use positive control |
| Too much background | Incubation time of Detection Solution or Blue Spot Solution too long;<br>Temperature during incubation too high | Check incubation time and temperature of Detection Solution and Blue Spot Solution |
| Strong, leaking signals | Incubation time of Detection Solution or Blue Spot Solution too long | Stepwise adjustment of the incubation time of Detection Solution and Blue Spot Solution |
| Weak signals | Hybridization temperature incorrect | Check temperature |
| | Hybridization time too short | Extend hybridization time |
| | Incubation time of Detection Solution or Blue Spot Solution too short | Extend incubation time of Detection Solution and Blue Spot Solution |
| 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 |

## 8 Explanation of the Symbols

| | |
|---|---|
|  | Check information material |
|  | Check Instruction for use |
|  | Manufacturer |
|  | Content sufficient for n tests |
|   | LOT number |
|   | Product code |
|  | Use until |
|  | Allowed storing temperature |
|  | Store in the dark |