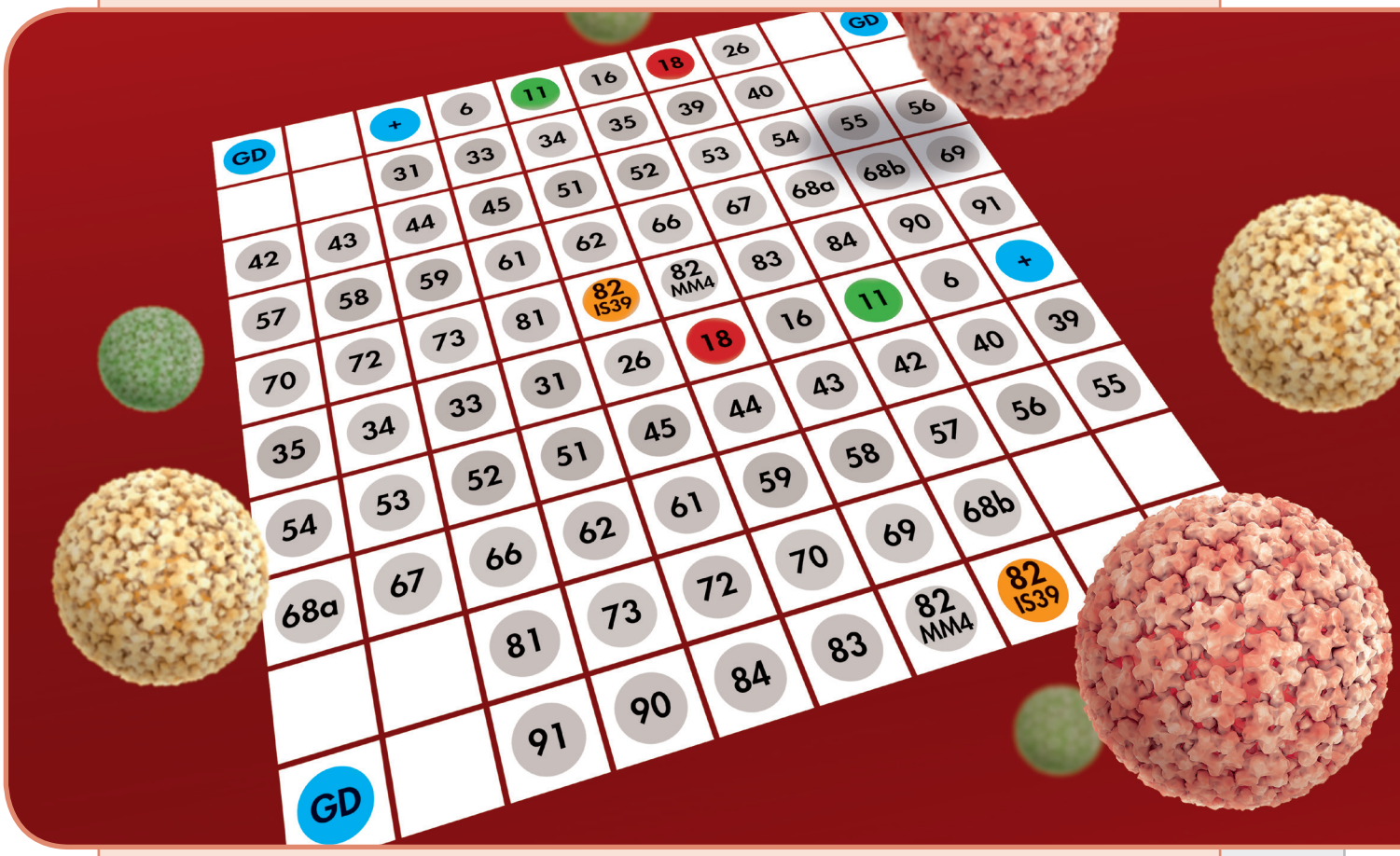
ZYTONEWS

VisionArray® HPV Chip 1.0

Results of the European HPV DNA Test External Quality Assurance Scheme (EHEQAS)



Mendel Center for Biomedical Sciences · www.mendelcenter.org/eheqas

Dear Readers,

With this new issue of our **ZYTONEWS** we would like to summarize the results of the **European HPV DNA Test External Quality Assurance Scheme (EHEQAS)** results achieved in April 2016 and May 2017. EHEQAS is an EQA scheme initiated in 2003 and formally founded in 2006. It already includes experts and diagnostic laboratories from Croatia, Cyprus, Czech Republic, Germany, Greece, Hungary, Netherlands, and Portugal.

Enjoy reading, Yours ZYTONEWS TEAM

ZYTOVISION
Molecular diagnostics simplified

Aim & Method of the Quality Testing

EHEQAS is an **External Quality Assurance Scheme** to assess and improve the quality of HPV DNA testing laboratories.

Any European laboratory performing HPV tests may participate. After registration the participants receive 5-7 samples for HPV detection 1-2 times per year. It is considered that participants should be able to report results to the coordinator within 3 weeks from receiving the samples. Subsequently, test reports of the results are evaluated by the coordinating team and the results and methods obtained from all participants are compared. Consensus results are issued and announced to the participants. Successful participation in EHEQAS is extremely helpful for the laboratories to verify and validate the results produced.

Overview of applied HPV Detection Methods

Test Assay	Method	Genotyping
Chipron HPV 3.5	PCR/reverse hybridization on LCD-Chip	✓
CLART® HPV	PCR/hybridization on a microarray surface, followed by an enzymatic colour reaction	✓
Digene HC2 HPV DNA Test	DNA-RNA hybrids are captured by antibodies, AP-detection with chemiluminescent substrate	–
INNO-LiPA® HPV Genotyping Extra II	PCR/reverse hybridization on membrane strips, followed by an enzymatic colour reaction	✓
VisionArray® HPV Chip 1.0	PCR/hybridization on the **VisionArray® HPV Chip**, followed by an enzymatic colour reaction	✓

Assessment of the Results

After receiving the test reports, the coordinating team is evaluating the results for each sample. Every tested sample can be assessed with a maximum of 10 marks:

- 5 of these 10 marks are awarded for a detection of the correct presence or absence of HPV DNA (Possible results: Negative, positive, insufficient sample, PCR inhibitor).
- The other 5 marks are awarded for a correct HPV genotyping. The correct HPV genotype as well as the detection of a possible multi-infection is of importance.

Results

Quality Control Trial I - April 2016

The results of 8 laboratories participating in EHEQAS April 2016 are summarized. Each laboratory received the same 5 or 7 EHEQAS samples (same requirements for all laboratories) which should be processed by applying their routine HPV testing method. If all 5 and 7 samples were evaluated successfully, the maximum score achievable was 50 and 70, respectively.
Two laboratories achieved the maximum score: Genomica HPV 3 and **VisionArray® HPV Chip 1.0** as HPV testing method.

Tab. 1: Overview of achieved scores for HPV testing, April 2016.

Lab	Method	Geno-typing	No. of Samples	Maximum Score	Achieved Score	% Score
A	Genomica HPV3 Microarrays	✓	7	70	69	98
B	HR hc2 Digene (Qiagen)	–	7	70	55	78
C	Genomica HPV3 Microarrays	✓	5	50	50	100
D	INNO-LIPA HPV genotyping Extra II	✓	5	50	30	60
E	Chipron HPV 3.5 LCD-Array Kit	✓	5	50	49	98
ZytoVision	**VisionArray® HPV Chip 1.0**	✓	**5**	**50**	**50**	**100**
G	INNO-LIPA HPV genotyping Extra II	✓	5	50	25	50
H	Chipron HPV 3.5 LCD-Array Kit	✓	5	50	33	66

Results: April 2016

Conclusion

ZytoVision VisionArray® HPV Chip 1.0 was used as testing method in 1 laboratory. ZytoVision achieved a full score of 50 marks (100%).

Results

Quality Control Trial II - May 2017

The results of 10 laboratories participating in EHEQAS May 2017 are summarized. Each laboratory received the same 5 or 7 EHEQAS samples (same requirements for all laboratories) which should be processed by applying their routine HPV testing method. If all 5 and 7 samples were evaluated successfully, the maximum score achievable was 50 and 70, respectively.
Two laboratories achieved the maximum score: InnoLipa HPV genotyping extra strip assay and **VisionArray® HPV Chip 1.0** as HPV testing method.

Tab. 2 Overview of achieved scores for HPV testing, May 2017.

Lab	Method	Genotyping	No. of Samples	Maximum Score	Achieved Score	% Score
A	HR hc2 Digene (Qiagen)	–	7	70	50	71
B	VisionArray® HPV Chip 1.0	✓	7	70	70	100
C	VisionArray® HPV Chip 1.0	✓	7	70	69	98
D	Chipron HPV 3.5 LCD-Array Kit	✓	7	70	50	71
E	INNO-LIPA HPV genotyping Extra II	✓	5	50	50	100
F	Chipron HPV 3.5 LCD-Array Kit	✓	5	50	48	96
ZytoVision	VisionArray® HPV Chip 1.0	✓	5	50	50	100
H	VisionArray® HPV Chip 1.0	✓	5	50	40	80
I	Chipron HPV 3.5 LCD-Array Kit	✓	5	50	44	88
J	VisionArray® HPV Chip 1.0	✓	5	50	48	96

Results: May 2017

Conclusion

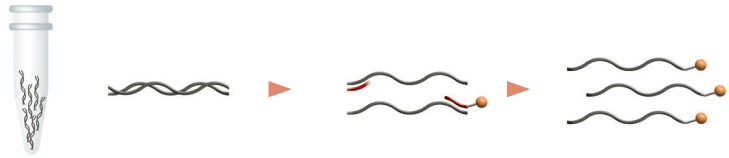
The **VisionArray® HPV Chip 1.0** was used as testing method in 5 laboratories. ZytoVision achieved a full score of 50 marks (100%). In 3 other laboratories using **VisionArray®** for testing, the obtained results were assessed as excellent (96-100%) demonstrating the high capability and reproducibility of the system.

Summary of Conclusions

- The **ZytoVision labaratory** participated successfully in EHEQAS in April 2016 and May 2017 with a **100% assessment**.
- **Other laboratories** started to use **VisionArray®** as testing method with **excellent EHEQAS results** (96-100%).
- The **VisionArray®** system reached **100% grading** in both EHEQAS HPV quality control trials, demonstrating the **high reliability, sensitivity and reproducibility** of this HPV test.

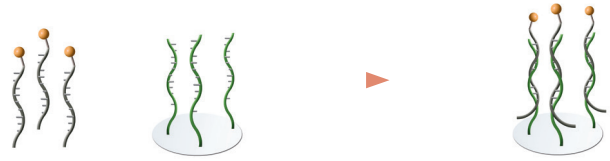
VisionArray® – Method Description

Step 1: Amplification and Labeling in a PCR



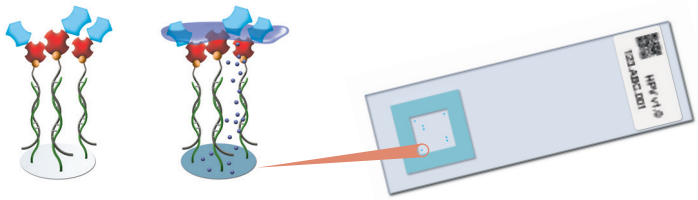
The DNA is extracted from e.g. FFPE samples and is used as a template for PCR. Biotinylated primers are used to amplify and label different sections of the target sequences. The human HLA-DQA1 gene is also amplified and serves as a PCR positive control and as a genomic control.

Step 2: Hybridization on the Glass Chip



After amplification, the biotinylated sequences hybridize to complementary DNA capture sequences on the glass chip.

Step 3: Detection and Visualization



Specifically bound and biotinylated sequences are visualized by secondary marking with a streptavidin-peroxidase conjugate and a staining with tetramethyl-benzidine. After color development, evaluation is performed using the **VisionArray® Analyzer Software**.

Product Information

Simultaneous genotyping of **41 different HPV types** – all certified for *in vitro* diagnostic use

- All capture sequences and positive controls are set up on the **VisionArray® HPV Chips** as duplicates
- High sensitivity and specificity
- Quick & easy 1 hour protocol
- Automated evaluation using the **VisionArray® Analyzer Software** – simple visualization & quick analysis in just a few minutes

GD		+	6	11	16	18	26		GD
		31	33	34	35	39	40		
42	43	44	45	51	52	53	54	55	56
57	58	59	61	62	66	67	68a	68b	69
70	72	73	81	82 IS39	82 MM4	83	84	90	91
35	34	33	31	26	18	16	11	6	+
54	53	52	51	45	44	43	42	40	39
68a	67	66	62	61	59	58	57	56	55
		81	73	72	70	69	68b		
GD		91	90	84	83	82 MM4	82 IS39		

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

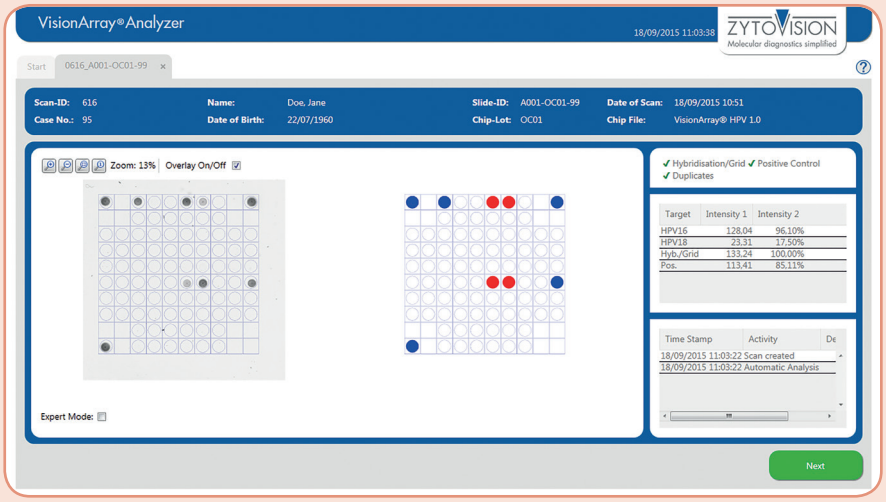
High Risk
Low Risk
Probably High Risk
Guide Dots (GD)/ Positive Control (+)

Prod. No.	Product	Tests
VA-0001-10	VisionArray HPV Chip 1.0 Incl. 10 pieces CE IVD	10
VA-0001-50	VisionArray HPV Chip 1.0 Incl. 5x 10 pieces CE IVD	50
VA-0002-10	VisionArray HPV High Risk Chip 1.0 Incl. 10 pieces CE IVD	10
VA-0002-50	VisionArray HPV High Risk Chip 1.0 Incl. 5x 10 pieces CE IVD	50

Product Information

VisionArray® Analyzer Software

- **Simple visualization** and **quick analysis** of the VisionArray® HPV Chip data
- **Analysis** of the VisionArray® HPV Chip and the report of the results can be achieved in **just a few minutes**
- **Program** navigation is very **easy** and **intuitive**
- Scans are stored in an **integrated database** on the enclosed external hard drive including all sample and chip data



Prod. No.	Product
E-4060-1	VisionArray Analysis Package CE IVD Incl. Scanner 8100; Slide Holder; Hand Scanner; PC with preinstalled VisionArray Analyzer Software; USB-Hub; External Hard Drive; Computer Mouse

VisionArray® Detection Kit

For hybridization and detection of PCR products on VisionArray® Chips

Prod. No.	Product	Tests
VK-0003-50	VisionArray Detection Kit CE IVD Incl. Hybridization Solution, 1 ml; Detection Solution, 5 ml; Blue Spot Solution, 5 ml; 100x Wash Buffer, 250 ml	50
E-4051-1	Mini Slide Centrifuge Incl. 2 place slide rotor, two slide holders	

VisionArray® DNA Extraction Kits

For isolation of genomic DNA from FFPE as well as liquid based cytology specimens

Prod. No.	Product	Tests
VI-0001-50	VisionArray FFPE DNA Extraction Kit Incl. Paraffin Dissolver; Tissue Lysis Buffer, Decrosslink Buffer; DNA Wash Buffer, Proteinase K; Proteinase K Buffer, Elution Buffer, Columns; Collection Tubes	50
VI-0002-50	VisionArray Cytology DNA Extraction Kit Incl. Pre-Lysis Buffer; Cell Lysis Buffer, DNA Wash Buffer, Proteinase K; Proteinase K Buffer, Elution Buffer, Columns; Collection Tubes	50

VisionArray® PCR Reagents

For contamination-free amplification and biotinylation of target sequences with a high quality heat stable Taq polymerase

Prod. No.	Product	Tests
VP-0001-50	VisionArray HPV Primer Kit CE IVD Incl. HPV Primer Mix; dNTP/dUTP Solution	50
VE-0001-100	VisionArray PreCise Taq DNA Polymerase CE IVD Incl. VisionArray PreCise Taq DNA Polymerase; Reaction Buffer, 10x; PreCise $MgCL_2$ 25 mM	100
VE-0002-100	VisionArray Uracil-DNA Glycosylase CE IVD Incl. VisionArray Uracil-DNA Glycosylase (UDG); UDG Reaction Buffer, 10x	100
ES-0007-50	VisionArray HPV PreCise Master Mix CE IVD	50

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