VisionArray FUNGI Chip 1.0

REF VA-0006-10 10 tests

For the specific detection of 30 fungi types that have been produced with the help of the VisionArray FUNGI PreCise Master Mix

For research use only.
Not for use in diagnostic procedures.

1. Intended purpose

The VisionArray FUNGI Chip 1.0 is intended to be used for the qualitative detection and genotyping of PCR-amplificates of 30 fungi genotypes that have been produced with the help of the VisionArray FUNGI PreCise Master Mix 1.0 (Prod. No. ES-0009-50) from formalin-fixed, paraffin-embedded specimens. The chip is intended to be used in combination with a VisionArray Software.

2. Test principle

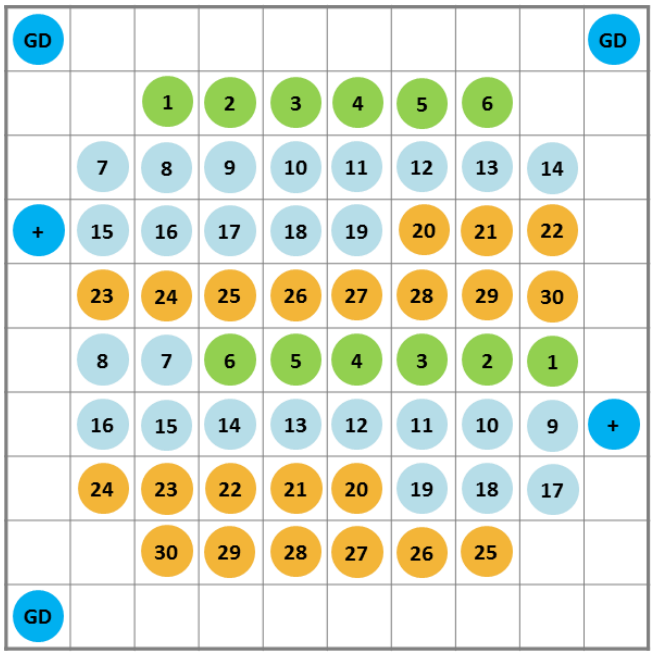
DNA-fragments with a specific sequence are detected from a pool of DNA-fragments on a glass chip with the help of immobilized DNA capture sequences by DNA/DNA-hybridization. For this detection system DNA-samples from formalin-fixed, paraffin-embedded tissue or cell samples can be used as raw material.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

3. Reagents provided

The following components are included:

Code	Components	Quantity
VA-0001	VisionArray FUNGI Chip 1.0	10
E-4206	VisionArray FUNGI Chip File 1.0	1
	Instructions for use	1

Description of the chip:
Positioning of the capture sequences on the chip:



GD	Guide Dot	15	Nakaseomyces glabratus
+	PCR positive control	16	Pichia fermentans
1	Aspergillus flavus	17	Pichia kudriavzevii
2	Aspergillus fumigatus	18	Pichia norvegensis
3	Aspergillus nidulans/quadrilineatus	19	Wickerhamomyces anomalus
4	Aspergillus niger	20	Cryptococcus neoformans
5	Aspergillus terreus	21	Fusarium spp.
6	Aspergillus versicolor	22	Lichtheimia corymbifera
7	Candida albicans	23	Mucor spp.
8	Candida auris	24	Paecilomyces variotii
9	Candida dubliniensis	25	Pneumocystis jirovecii
10	Candida parapsilosis	26	Purpureocillium lilacinum
11	Candida tropicalis	27	Rhizomucor pusillus
12	Clavispora lusitaniae	28	Rhizopus spp.
13	Kluyveromyces marxianus	29	Scedosporium spp.
14	Meyerozyma guilliermondii	30	Trichophyton/Microsporum

Description of the Chip File:
Please download the VisionArray FUNGI Chip File 1.0 using the following link:

https://www.zytovision.com/chip-file/e-4206.zip

4. Materials required but not provided

- VisionArray SingleScan Software (E-4301) or VisionArray MultiScan Software (E-4302)
- VisionArray FUNGI PreCise Master Mix 1.0 (ES-0009)
- VisionArray Detection Kit (VK-0003)

The VisionArray Softwares have to contain the VisionArray FUNGI Chip File 1.0 (E-4206) for a successful scan.

5. 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. Return to storage conditions immediately after use. Do not use reagents beyond expiry date indicated on the label. The product is stable until expiry date indicated on the label when handled accordingly.

6. Warnings and precautions

- Read the instructions for use prior to use!
- Do not use the products after the expiry date has been reached!
- Please check if packaging is intact before use, do not use product if packaging is damaged.
- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!
- If reagents come into contact with skin, rinse skin immediately with copious amounts of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse products, unless reuse is explicitly permitted!
- Avoid cross-contamination of samples as this may lead to erroneous results.
- A room separation of working steps with and without DNA as well as using clean benches for preparation of the PCR master mix is necessary to avoid contaminations.
- 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.

Hazard and precautionary statements:

This product is not classified as hazardous according to Regulation (EC) No. 1272/2008.

7. Limitations

- For research use only.
- For professional use only.
- For non-automated use only.

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 fungi infections or in case of multiple infections the intensity of the PCR 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.

8. Interfering substances

- Low PCR efficiency due to PCR inhibitors in DNA raw material (e.g. blood).
- High concentrations of EDTA in DNA elution buffers may lead to an inhibition of the PCR. Use only the recommended amounts of DNA.
- Use of PCR additives that could influence the hybridization (e.g. DMSO, betaine, urea).

9. Preparation of specimens

Starting material for this detection system are PCR amplification products that have been produced with the Vision*Array* FUNGI PreCise Master Mix 1.0.

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

10. Preparatory treatment of the device

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

11. Assay procedure

Perform the scan according to the instructions for use of the respective Vision*Array* Software.

12. Interpretation of results

With the aid of the Vision*Array* FUNGI Chip 1.0, it is possible to make a qualitative statement about the presence or absence of one or more of 30 fungi 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 respective Vision*Array* Software. A comprehensive manual for a chip-analysis is enclosed to the Software.

13. Recommended quality control procedures

Internal controls:

- Guide dots/Hybridization control (GD): These dots are used by the respective Vision*Array* 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.

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 any of the internal and/or external controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

14. Performance characteristics

14.1 Analytical performance

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 respective Vision*Array* Software.

15. Disposal

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

16. 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 DNA 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 fungi Signals, but no positive control	Strong fungi infection or multiple fungi 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

17. Literature

- Kidd S, et al. (2023) *Open Forum Infect Dis*;10(1):ofac559. doi:10.1093/ofid/ofac559. PMID: 36632423; PMCID: PMC9825814.
- Schoch CL, et al. (2012) *Proc Natl Acad Sci U S A.*;109(16):6241-6. doi: 10.1073/pnas.1117018109. PMID: 22454494; PMCID: PMC3341068.

18. Revision

www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use as well as for instructions for use in different languages.

Our experts are available to answer your questions.
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