VisionArray MYCO Chip 2.0

REF VA-0005-10 Σ 10 tests

For the specific detection of genus Mycobacterium and several clinically relevant mycobacterial species that have been produced with the help of the VisionArray MYCO PreCise Master Mix 2.0

CE IVD

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

1. Intended use

The VisionArray MYCO Chip 2.0 is intended to be used with a VisionArray Software for the qualitative detection and identification of PCR-amplificates of the genera Mycobacterium, Mycobacteroides, Mycolicibacillus, Mycolicibacter and Mycolicibacterium as well as several clinically relevant mycobacterial species therein that have been produced with the help of the VisionArray MYCO PreCise Master Mix 2.0.

The genus Mycobacterium has recently been divided into 5 distinct genera Mycobacterium, Mycobacteroides, Mycolicibacillus, Mycolicibacter and Mycolicibacterium as proposed by Gupta et al. (2018) and validly published in *Validation List No. 181* (Oren and Garrity 2018). However, for the sake of clarity and consistency, the former single genus mycobacterium will still be used in parts of this manual.

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

The emended mycobacterial genera comprise more than 140 species, which, for the purpose of diagnosis and treatment, have been grouped into three categories: M. tuberculosis complex (MTC), M. leprae, and nontuberculous mycobacteria (NTM). Due to their differences in pathogenicity, virulence, and response to drugs, the detection and differentiation of these pathogens in clinical specimens of patients with clinically suspected tuberculosis is of uttermost importance.

The VisionArray MYCO Chip 2.0 is designed to detect the mycobacterial species shown in chapter 4 "Reagents provided".

3. Test principle

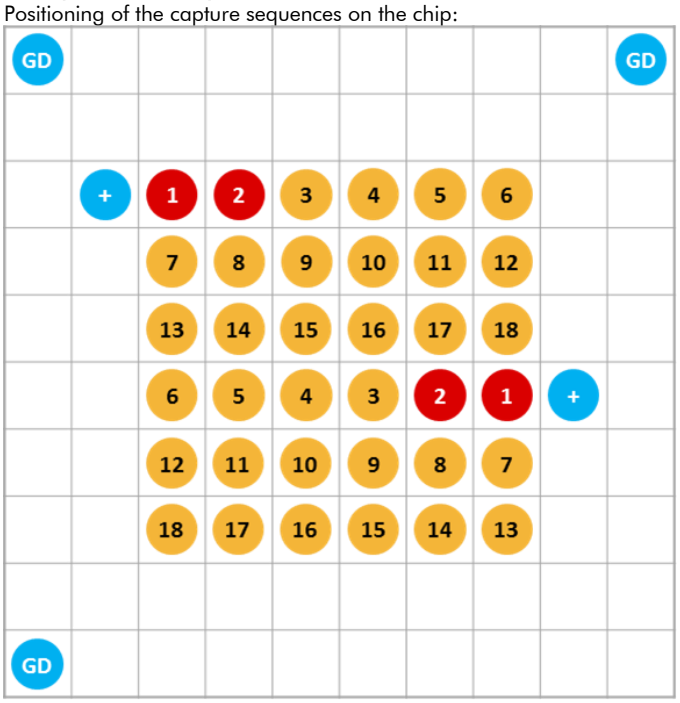
DNA fragments with a specific sequence are detected from a pool of DNA fragments on a glass chip with the help of immobilized DNA capture sequences by DNA/DNA-hybridization. For this detection system DNA samples extracted from e.g. clinical specimens, pulmonary smears or cultivated samples can be used 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 capture sequences is performed subsequently. After the hybridization, the unspecifically bound DNA is washed away by short stringent wash steps. The specific bound biotinylated sequences are secondarily 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 Σ
VA-0005	VisionArray MYCO Chip 2.0	10
	Instructions for use	1

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



GD	Guide Dot	9	M. gordonae
+	PCR positive control	10	M. haemophilum
1	M. tuberculosis complex (ITS Region)	11	M. kansasii
2	M. tuberculosis complex (IS6110 Region)	12	M. malmoense
3	M. abscessus	13	M. marinum / M. ulcerans
4	M. avium / M. intracellulare complex	14	M. scrofulaceum / M. parascrofulaceum
5	M. chelonae	15	M. simiae
6	M. chimaera	16	M. smegmatis
7	M. fortuitum	17	M. szulgai
8	M. genavense	18	M. xenopi

The dot for the M. tuberculosis complex comprises: M. tuberculosis, M. bovis, M. africanum, M. caprae, M. microti, and M. pinnipedii.

The dot for the M. avium / M. intracellulare complex comprises: M. avium, M. chimaera, M. arosiense, M. timonense, M. yongonense, M. lepraemurium, M. intracellulare, M. colombiense, M. bouchedurhonense, M. marseillense, and M. paraintracellulare.

Description of the Chip File:
Please download the VisionArray MYCO Chip File 2.0 using the following link:
https://www.zytovision.com/chip-file/e-4205.zip

5. Materials required but not provided

- VisionArray SingleScan Software (E-4301) or VisionArray MultiScan Software (E-4302)
- VisionArray MYCO PreCise Master Mix 2.0 (ES-0008)
- VisionArray Detection Kit (VK-0003)

The VisionArray Softwares have to contain the VisionArray MYCO Chip File 2.0 (E-4205) 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 instructions for use prior to use!
- Please check if packaging is intact before use, do not use product if packaging is damaged.
- 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 mycobacterial species 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 mycobacterial 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.

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 VisionArray MYCO PreCise Master Mix 2.0.

The hybridization and detection of the chips has to be performed with the VisionArray 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 instructions for use of the respective VisionArray Software.

13. Interpretation of results

With the aid of the VisionArray MYCO Chip 2.0, it is possible to make a qualitative statement about the presence or absence of one or more of mycobacterial species 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 VisionArray 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 respective VisionArray 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.

15. Performance characteristics

15.1 Analytical performance

Analytical specificity and sensitivity of the VisionArray MYCO Chip 2.0 was tested for each mycobacterial species on the chip separately. For this purpose sequence verified plasmids with a concentration of 5-500,000 genome equivalents (GEQ) were tested.

Specificity and limit of detection for the mycobacteria species

Mycobacterium	Specificity [%]	Limit of Detection (GEQ)
M. tuberculosis complex (ITS)	100	50
M. tuberculosis complex (IS6110)	100	5
M. abscessus	100	500
M. avium / M. intracellulare complex	100	50
M. chelonae	100	1000
M. chimaera	100	50
M. fortuitum	100	50
M. genavense	100	50
M. gordonae	100	50
M. haemophilum	100	50
M. kansasii	100	50
M. malmoense	100	50
M. marinum / M. ulcerans	100	50
M. scrofulaceum / M. parascrofulaceum	100	50
M. simiae	100	50
M. smegmatis	100	50
M. szulgai	100	50
M. xenopi	100	50

The sensitivity depends on the amount and efficiency of the PCR-cycles and the affinity of the DNA capture sequences.

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 mycobacterial species, 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 instructions for use. Modifications to these procedures might alter the performance and have to be validated by the user.

15.2 Cross hybridizations

No Cross hybridizations were observed when tested with a concentration of up to 500,000 GEQ.

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 respective Vision*Array* 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 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 MYCO Signals, but no positive control	Strong Mycobacteria infection or multiple Mycobacteria infection	Dilute sample DNA
	Mycobacteria cell culture material, no human genomic DNA in sample	-
	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
Crosshybridization 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

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- Simons S., et al (2011) Emerg Infect Dis. 17(3):343-9
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Our experts are available to answer your questions.
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