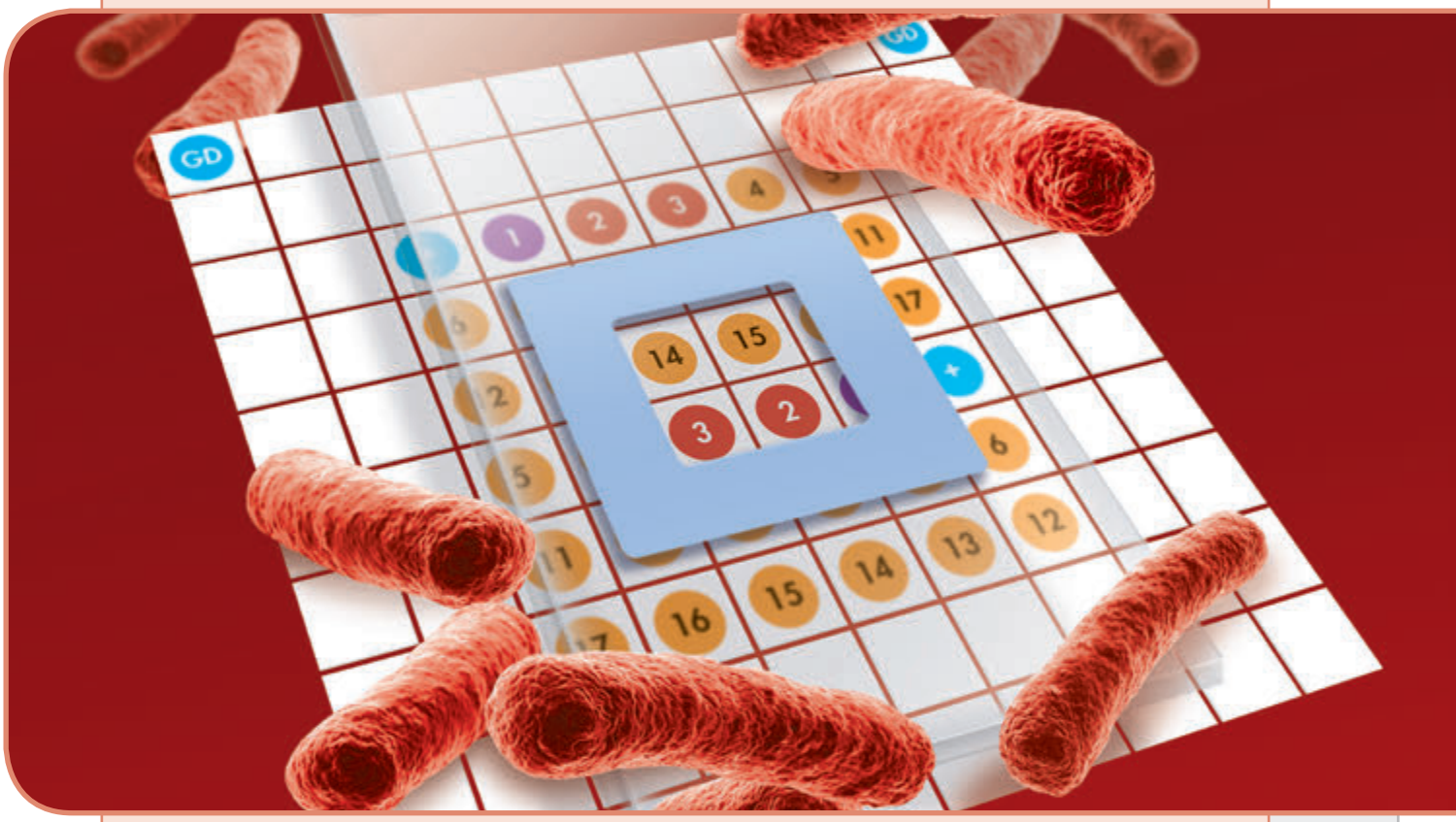
ZYTONEWS

VisionArray® MYCO Chip 1.0

Results of the »TBC-PCR« comparison test conducted by the Quality Assurance Initiative Pathology (QuIP, Qualitätssicherungs-Initiative Pathologie)



QuIP TBC-PCR · www.quip.eu

Dear Readers,

With this new issue of our **ZYTONEWS** we would like to summarize the results of the ring trial "TBC-PCR" performed in 2019 by the Qualitätssicherungs-Initiative Pathologie (QuIP, Quality Assurance Initiative Pathology). Since 2004, the QuIP has been offering roundrobin testing services for histopathological, immunohistological and molecular-pathological methods for quality assurance purposes.

Enjoy reading, Your ZYTONEWS TEAM

ZYTOVISION
Molecular diagnostics simplified

Causative Agents of Tuberculosis

Tuberculosis (TBC) is the leading cause of death among infectious diseases from a single agent, as well as the ninth leading cause of death worldwide. It is easily acquired through the inhalation of infected respiratory droplets. In 2018, around 10 million people developed TBC and 1.2 million died as a result of this disease. TBC is caused by members of the Mycobacterium tuberculosis complex (MTC) comprising ten different Mycobacterium species.
Rapid and reliable identification of mycobacterial infections is critical in guiding public health and primary care decisions due to the species-dependent differences in epidemiology, geographic range, pathogenicity, and drug susceptibility.
While microscopic examination and culturing continue to be indispensable for laboratory diagnosis of TBC, the range of several molecular diagnostic tests has expanded tremendously. These tests provide timely results useful for high-quality patient care.

Aim of the MTC Quality Testing Scheme

The **Quality Assurance Initiative Pathology (QuIP)**, as a joint venture of the German Society for Pathology e.V. (DGP) and the Federal Association of German Pathologists e.V., offers ring trials for pathological institutes and methods for quality assurance purposes. It supports pathologists in optimizing their examination methods and results. QuIP offers interlaboratory comparisons for immunohistochemical and molecular pathological methods as external control.

The **TBC-PCR** ring trial is conducted every second year by QuIP with several laboratory specific molecular methods for the detection of DNA from Mycobacterium species in formalin-fixed paraffin-embedded (FFPE) tissues. The comparison of the results allows statements to be made about the diagnostic accuracy in general or about the quality of the results of the participating practices and institutes.

Assessment Method

- Each participating laboratory receives ten different FFPE specimens which must be analyzed by a molecular method.
- For each specimen, the results, indicating whether DNA from members of the M. tuberculosis complex (MTC-DNA) is present in the test material (yes/no), must be submitted to the QuIP within 10 days from receiving the samples.
- After receiving the test reports, the coordinating team evaluates the results for each sample and compares them to the results obtained by the reference laboratories. Every tested sample can be assessed with a maximum of 2 points, resulting in a maximum score of 20 points for all ten specimens. If a case cannot be evaluated (e.g. due to difficult material), 1 point is awarded. This possibility can only be used once. For a successful participation, the laboratories must achieve a total score of at least 19 points.

References:
Chin KL, et al. (2018) Tuberculosis (Edinb) 113: 139-152.
Eddabra R & Ait Benhassou H (2018) Pneumonia (Nathan) 10: 4.
Ruiz-Tagle C, et al. (2020) Infect Immun 88: e00649-19.

Results

35 laboratories participated in the 2019 QuIP TBC-PCR ring trial using different molecular methods for the detection of DNA from Mycobacterium species that can cause tuberculosis. The expected results are shown in Tab. 1.

Tab. 1: Testing material indicating the results obtained by the reference laboratories.

Sample	1	2	3	4	5	6	7	8	9	10
Tissue	tongue	testis	lung	lung	eye	lung	lung	lung	aortic wall	lung
MTC-DNA?	yes	–	–	–	–	–	–	yes	yes	–

Part I - Detection of DNA from Mycobacterium species

Tab. 2: Overview of the results of all participating laboratories.

	Sample	1	2	3	4	5	6	7	8	9	10
No. of Labs	MTC-DNA detected	34	0	2	1	2	1	1	34	35	0
	MTC-DNA not detected	0	35	32	33	33	34	34	1	0	30
	Technically not possible	1	0	1	1	0	0	0	0	0	5

Red numbers indicate the result obtained by ZytoVision with the VisionArray® MYCO Chip 1.0 among other methods.

Part II - Final Results of the participating Testing Systems

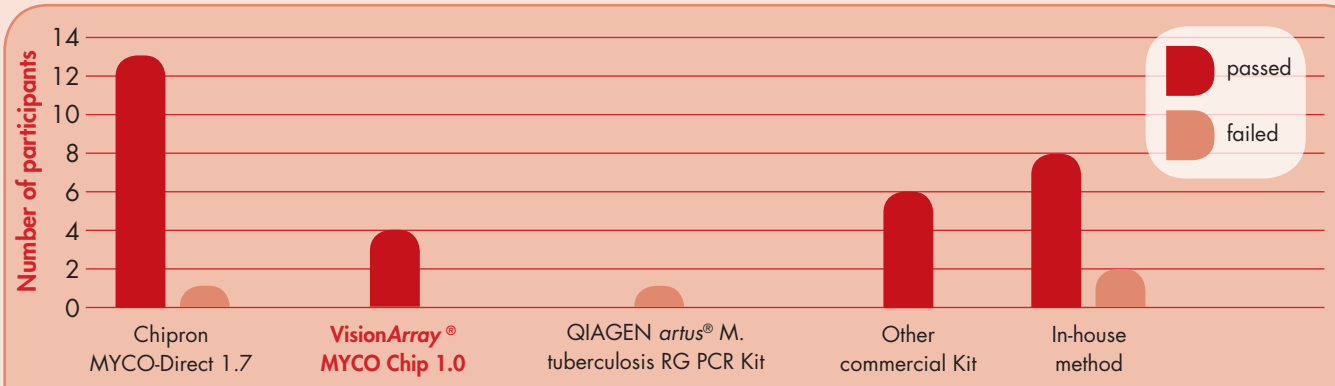


Fig. 1: MTC-DNA testing methods of participating laboratories and the results obtained with these methods.

Summary of the Results

- 31 laboratories (89%) participated successfully in the TBC-PCR program by achieving at least 19 out of 20 points.
- The **VisionArray® MYCO Chip 1.0** was used successfully as testing method in 4 laboratories including the ZytoVision laboratory.
- ZytoVision achieved a full score of 20 points.

VisionArray® – Method Description

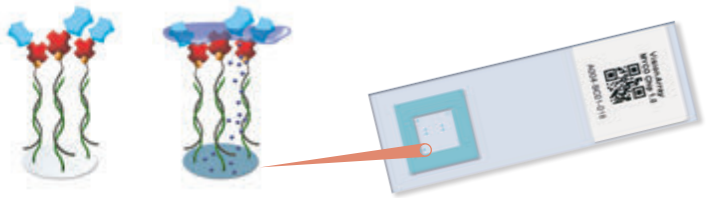
Step 1: Amplification and Labeling in a PCR

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

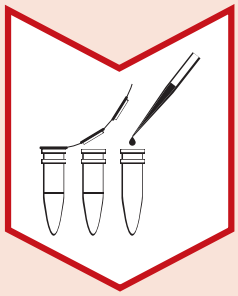


Specifically bound and biotinylated sequences are visualized by secondary marking with a streptavidin-peroxidase conjugate and a staining with tetramethyl-benzidine. After color development, evaluation is performed using a **VisionArray® Analyzer Software**.

VisionArray® – Workflow Schedule

This is a condensed protocol for the VisionArray® method and should not replace the instruction for use!

0 Min.



PCR

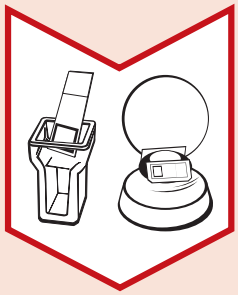
- For the PCR the ready-to-use VisionArray® MYCO PreCise Master Mix is used
- The VisionArray® MYCO PreCise Master Mix contains the components of the VisionArray® MYCO Primer Kit 1.0, the VisionArray® PreCise Taq DNA Polymerase, and the VisionArray® Uracil-DNA Glycosylase
  All reagents can be ordered separately
- DNA sample is added to the master mix

30 Min.

Hybridization

- PCR product and Hybridization Solution are mixed well
- Mix is applied onto the VisionArray® Chip

Duration: 30 min

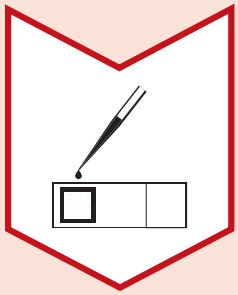


Stringency Wash

- Unbound DNA fragments are removed using 1x Wash Buffer
- Drying of VisionArray® Chip by centrifugation

Duration: 2 min

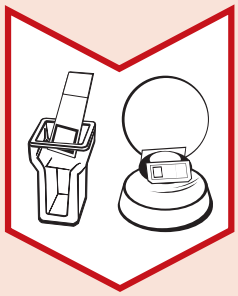
50 Min.



Detection

- Marking of biotinylated sequences using the Detection Solution
- Visualization is performed by applying the Blue Spot Solution

Duration: 17 min

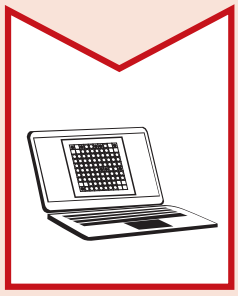


Wash

- Removing of the Blue Spot Solution by washing with 1x Wash Buffer
- Drying of VisionArray® Chip by centrifugation

Duration: 2 min

60 Min.



Analysis

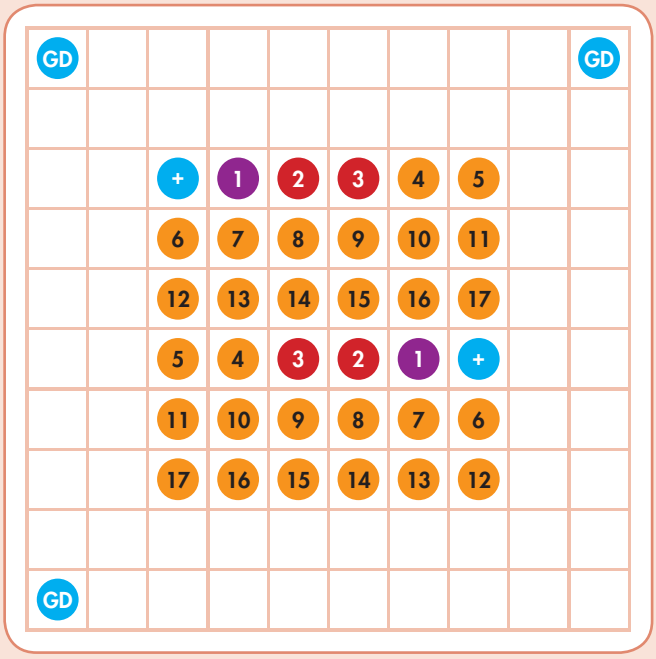
- Chips are scanned with the VisionArray® Scanner 8100 or the VisionArray® Scanner V600 Photo
- Automated analysis is performed by using a VisionArray® Analyzer Software

Duration: 10 min

Quick & easy 1 hour protocol · Automated user-friendly evaluation within a few minutes

Product Information

- Simultaneous genotyping of the most relevant different Mycobacterium species – all certified for *in vitro* diagnostic use
- All capture sequences and positive controls are set up on the **VisionArray® Chips** as duplicates
- High sensitivity and specificity
- One patient sample - one slide
- Quick & easy 1 hour protocol
- Automated evaluation using a **VisionArray® Analyzer Software** – simple visualization & quick analysis in just a few minutes



M. tuberculosis (MTC) complex

2	M. tuberculosis complex (ITS Region)
3	M. tuberculosis complex (IS6110 Region)

Nontuberculous Mycobacteria (NTM)

4	M. abscessus	11	M. malmoense
5	M. avium / M. intracellulare complex	12	M. marinum / M. ulcerans
6	M. chelonae	13	M. scrofulaceum / M. parascrofulaceum
7	M. fortuitum	14	M. simiae
8	M. genavense	15	M. smegmatis
9	M. haemophilum	16	M. szulgai
10	M. kansasii	17	M. xenopi

Prod. No.	Product	Tests
VA-0003-10	VisionArray MYCO Chip 1.0 Incl. 10 pieces CE IVD NEW	10
VA-0003-50	VisionArray MYCO Chip 1.0 Incl. 5x 10 pieces CE IVD NEW	50

CE IVD *only available in certain countries. All other countries research use only! Please contact your local dealer for more information.*

Product Information

Software-Based Analysis

- **Simple visualization** and **quick analysis** of the Vision*Array*® MYCO Chip data
- **Analysis** of the Vision*Array*® MYCO 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

VisionArray ® Analysis Package SingleScan

VisionArray ® Analysis Package MultiScan

Prod. No.	Product
E-4060-1	Vision*Array* Analysis Package SingleScan CE IVD Incl. Scanner 8100; Slide Holder SingleScan; Hand Scanner; PC with preinstalled Vision*Array* Analyzer Software SingleScan; USB-Hub; External Hard Drive; Computer Mouse
E-4070-1	Vision*Array* Analysis Package MultiScan CE IVD Incl. Scanner V600 Photo; Slide Holder MultiScan; PC with preinstalled Vision*Array* Analyzer Software MultiScan; USB-Hub; External Hard Drive; Computer Mouse

Vision*Array*® Detection Kit

For hybridization and detection of PCR products on Vision*Array*® Chips

Prod. No.	Product	Tests
VK-0003-50	Vision*Array* 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	

Vision*Array*® DNA Extraction Kits

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

Prod. No.	Product	Tests
VI-0001-50	Vision*Array* 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	Vision*Array* 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

Vision*Array*® PCR Reagents

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

Prod. No.	Product	Tests
ES-0008-50	Vision*Array* MYCO PreCise Master Mix CE IVD Containing MYCO Primer Mix 1.0; dNTP/dUTP Solution; Vision*Array* PreCise Taq DNA Polymerase; PCR-Buffer; $MgCl_2$; Vision*Array* Uracil-DNA Glycosylase	50
Related Products		
VP-0002-50	Vision*Array* MYCO Primer Kit 1.0 CE IVD Incl. MYCO Primer Mix 1.0; dNTP/dUTP Solution	50
VE-0001-100	Vision*Array* PreCise Taq DNA Polymerase CE IVD Incl. Vision*Array* PreCise Taq DNA Polymerase; PreCise Reaction Buffer, 10x; PreCise $MgCl_2$, 25 mM	100
VE-0002-100	Vision*Array* Uracil-DNA Glycosylase CE IVD	100

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