Submission information for Vista ctDNA test family

1 Short description

The **Vista** family of products are in-house ctDNA sequencing tests developed by LIQOMICS. These tests are designed for exploratory genotyping in cancer patients using liquid biopsy samples and to detect minimal residual disease (MRD) in follow-up samples.

Examples are **LymphoVista**, a test for genotyping and MRD detection in lymphoma, **LymphoVista HL**, a specific **LymphoVista** variant for genotyping and MRD detection in Hodgkin lymphoma, and **CancerVista**, a test for genotyping and MRD detection in solid tumors. The products are in-house in vitro diagnostic (IVD) send-in services and include the entire workflow from cell-free DNA (cfDNA) extraction, quality control, sequencing library preparation, target enrichment and sequencing, followed by the analysis with an in-house pipeline.

2 Regulatory framework

We currently offer the **Vista** family of tests as in-house IVD tests in a quality-controlled environment after validation and according to the German RiliBÄK guidelines. Currently, **CancerVista**, **LymphoVista** and **LymphoVista HL** are offered under this regulatory framework.

3 Analyte

The analyte is cfDNA, collected in special collection tubes that separate and preserve the cellular fraction of the collected blood so that it can be cleanly separated from the plasma in the LIQOMICS laboratory. We mainly use Sarstedt cfDNA tubes (S-Monovette® cfDNA Exact, CE-IVD, 9.2 ml, cap raspberry colored, Sarstedt, 01.2040.001), but also accept Qiagen ccfDNA tubes (PAXgene Blood ccfDNA Tube (CE-IVD), Qiagen, 768165), as well as Strecktubes (Cell-Free DNA BCT® CE (10 ml, CE-IVD), Streck, 218996) for blood collection.

The Sarstedt cfDNA tubes are available upon request from LIQOMICS in form of a **Vista collection kit**, containing the cfDNA tubes and all instructions you need, to send the samples to LIQOMICS (see Chapter 8). Other forms of genetic material, such as previously extracted cfDNA or cfDNA from cerebrospinal fluid or urine, can sometimes be analyzed, but the test is not yet validated and results are provided for research purposes only. Please contact us to discuss other cfDNA sources if you are interested in analyzing them.

4 Indications and diseases for which the Vista family tests can be used

The indications for the **Vista** tests vary by test type. The **LymphoVista** test is designed for B-cell lymphomas, including splenic B-cell lymphoma, marginal zone lymphoma, follicular lymphoma, transformed indolent B-cell lymphoma, large B-cell lymphoma, CNS lymphoma, Burkitt's lymphoma, and KSHV/HHV8-associated B-cell lymphoid proliferations. For Hodgkin's lymphoma, we offer the **LymphoVista HL** test, a specialized version of our **LymphoVista** test. While the **LymphoVista** test could be used, we recommend the **LymphoVista HL** for Hodgkin lymphoma as it is specifically designed to include a broader range of genes relevant to this disease.The **CancerVista** test detects mutations in a range of solid tumors, including skin-, lung-, breast-, colorectal- and prostate cancer.

5 Test function and purpose

The **LymphoVista** test can be used to genotype lymphomas and monitor disease progression by assessing MRD. In certain cases, such as Hodgkin lymphoma and diffuse large B-cell lymphoma, MRD levels have shown prognostic value (see Chapter 6 for details). The **Vista** tests can be used to monitor disease progression and response to treatment, aiding in ongoing patient care. A quantitative result is provided in form of an absolute MRD level that can be compared to other time points where the test has been used. The tests should not be used for screening or diagnosis.

6 Analytical and Clinical Performance

6.1 LymphoVista

Technical validation [1]:

- **Variant Detection:** 93.86% sensitivity and 99.999% specificity for variants with ≥0.5% mutated allele frequency (mAF)
- **MRD Assessment:** Limit of Detection (LoD): 6.69×10^{-6}; specificity, sensitivity and accuracy: 100% for MRD levels $>3.04\times10^{-5}$.

Clinical validation (Vista platform, rrDLBCL) [2]:

The clinical validation study included 326 samples from 88 patients across 131 treatment lines. Patients who achieved MRD-negativity at any time point had a significantly higher 18-month overall survival (OS) of 77% compared to 33% for patients who were never MRD-negative (HR 4.61, 95% CI 2.38-8.92, p<0.0001). 18-month progression-free survival (PFS) was 51% for MRD-negative versus 5% for MRD-positive cases (HR 4.32, 95% CI 2.46-7.61, p<0.0001). This suggests that MRD can be used as an independent predictor of poor outcome in the absence of MRD-negativity.

6.2 LymphoVista HL

Technical validation [3]:

- **Variant Detection:** 91.27% sensitivity and 99.99% specificity for variants with ≥0.5% mAF.
- **MRD Assessment:** LoD: 6.54×10^{-6}; specificity, sensitivity and accuracy: 100% for MRD levels $>1.76\times10^{-5}$.

Clinical validation (LymphoVista HL, HL) [3]:

The clinical validation study was based on patients from the HD21 trial with a median follow-up of 50 months. Here, the MRD positive rate after 2 cycles of chemotherapy (MRD-2 positive rate) was 18.5%. The 4-Year PFS was significantly higher in MRD-2 negative patients (95.3%) as compared to MRD-2 positive patients (72.2%) (HR 6.9, p<0.0001).

In subgroup analyses, MRD-2 remained prognostic for the outcome in PET-2 positive/negative patients, as well as patients in treatment-specific cohorts (e.g., BrECADD, eBEACOPP).

6.3 CancerVista

Technical validation [4]:

- **Variant Detection:** 93.17% sensitivity for variants with ≥1% mAF and >99% specificity for all variants.
- **MRD Assessment:** LoD: 3.30×10^{-6}, threshold at which MRD positivity is called: 2.5×10^{-5} (25ppm), specificity of 96.7%, sensitivity of 80% and accuracy of 94.3% for MRD levels $>5.1x10^{-5}$.

7 Principle of testing

The **Vista** family of tests can be used to genotype a cancer by liquid biopsy / tumor-derived cell-free DNA (ctDNA) sequencing. By combining results from a baseline sample (taken prior to treatment initiation) and one or any number of samples taken at a later time point, residual disease can be determined by tracking residual ctDNA quantified from tumor-specific mutations. First, cfDNA is extracted. After quality control of the cfDNA, sequencing libraries are prepared and enriched for the relevant targets of each **Vista** assay. The libraries are sequenced on an Illumina sequencer. The resulting data is analyzed using LIQOMICS' proprietary genotyping and MRD pipeline and results are reported in a standardized format.

7.1 Sample prerequisites

The first sample from a patient should be collected in a state of active disease to identify as many markers (mutations) as possible to track MRD. Subsequent samples can be collected at any time, even in the absence of visible disease (e.g., as indicated by medical imaging) to identify MRD that may not be visible on an imaging scan such as computed tomography (CT) or positron emission tomography (PET).

7.2 When the test is not possible

Patients with a previous allogeneic stem cell or organ transplant (except corneal transplant) cannot be tested.

7.3 Test limitations

The **Vista** family of tests should not be used for cancer screening. The primary function of genotyping with **Vista** family tests is to identify variants for MRD tracking and may miss certain cancer genotypes, especially in situations of low ctDNA content in a sample. While genotyping and MRD detection have been validated for the Vista family of tests, genotyping should currently not be used to qualify a patient for a specific treatment that would require a specific cancer genotype.

8 Sample collection, storage and shipment

This chapter provides detailed instructions on how to collect, store, and ship blood samples to LIQOMICS. Please read these guidelines carefully to ensure sample integrity.

Although we accept additional types of cfDNA tubes (see Chapter 3), we prefer to use the Sarstedt S-Monovette® cfDNA Exact tubes. These tubes are the ones provided in our **Vista collection kit** and their usage is described in detail in this section.

8.1 The LIQOMICS Vista collection kit

To facilitate blood sample collection and ensure proper handling, we provide the **Vista collection kit**, a cfDNA collection kit that includes all necessary materials. This kit is supplied in an environmentally friendly, reusable box, which can also be used to return the collected samples. To obtain a kit, please contact us via our webpage, e-mail, or phone (see Chapter 11). Upon request, we will provide the kit, along with all necessary instructions.

Kit Contents: Each box contains the following items:

- **Return package:** This is the box you received your kit in – please keep it!
  Note: If sending multiple samples in one box please **label each sample clearly** and provide an accompanying **submission sheet for each sample**, otherwise the samples cannot be processed.
- **Sarstedt cfDNA tube:** S-Monovette® cfDNA Exact (9.2 ml, **raspberry-colored cap**, Sarstedt, Cat. No. 01.2040.001) – please only use **this tube**!
- **Empty/ discard tube:** S-Monovette® neutral Z (4.9 ml, white cap, Sarstedt, Cat. No. 04.1926.001)
- **Sarstedt cfDNA tube product sheet:** contains the information on how to extract blood using the Sarstedt S-Monovette® cfDNA Exact tubes
- **Venous puncture set and adapter:** Safety-Multifly® Kanüle 21G (green, 200 mm, Sarstedt, 85.1638.235) – you can substitute this by any suitable venous puncture set with adapter
- **Sample container:** Transparent protection vessel, cylindrical design with absorbent insert (126 mm length, 30 mm opening diameter, Sarstedt, Cat. No. 78.898) – needed to protect against accidental sample spillage during transport to LIQOMICS' laboratory
- **Sample container lid:** Screw cap, natural color, compatible with the transport vessel (Sarstedt, Cat. No. 65.679)
- **Submission sheet:** To be completed, signed, and returned with the samples – **analysis without the fully completed sheet is not possible**!
- **This information leaflet:** General and sample processing information for the LIQOMICS **Vista** family of tests
- **Return label:** Prepaid label for easy sample return to LIQOMICS' laboratory

Note: Please read the accompanying instructions carefully to ensure proper use of the kit and the integrity of your samples during transit. For any questions, do not hesitate to reach out to us using the provided contact details (see Chapter 11).

8.2 Sample collection

1. Inform the patient and complete documentation
Please ensure that the patient is informed about all relevant details outlined in the consent and submission form. The form must be completed and signed before sample collection.

2. Blood collection using Sarstedt cfDNA tubes
When collecting blood samples, use Sarstedt S-Monovette® cfDNA Exact tubes with the raspberry-colored cap. For detailed guidance on the blood collection procedure, refer to the Sarstedt S-Monovette® cfDNA Exact product sheet included in the kit.
Do not freeze the blood samples! Proper handling and temperature control are critical to maintain sample integrity. After collection, please follow the instructions in the kit for packaging and shipping (Chapter 8.3 and Illustration 1), using the return box and label provided.

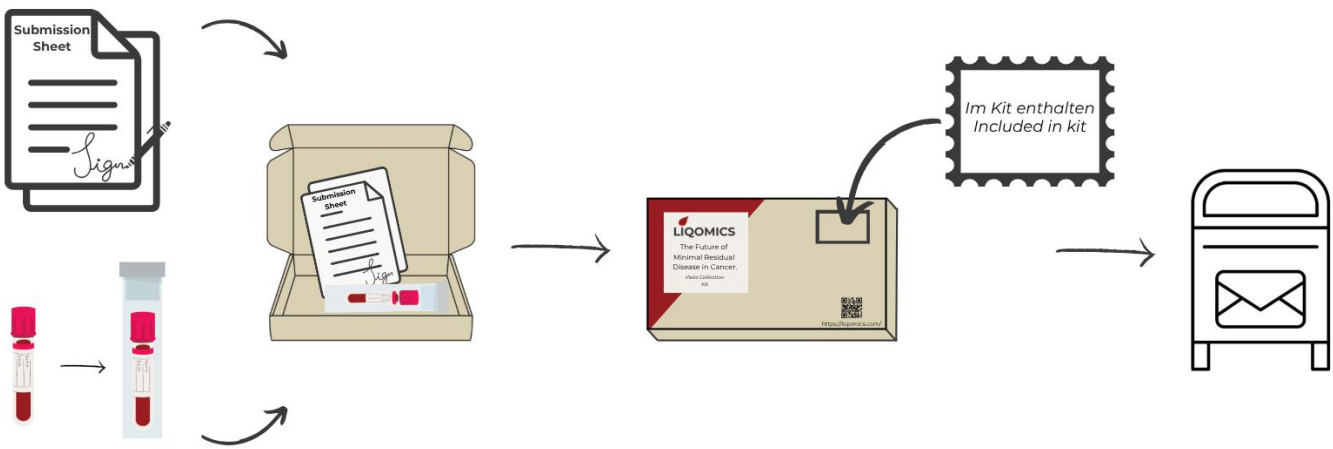
3. Special cases
If you are unable to follow the outlined procedure or have alternative materials, please contact us (see Chapter 11). We will work with you to find an individualized solution to accommodate your needs.

8.3 Sample storage and shipping

1. Storage of cfDNA in Sarstedt cfDNA tubes
cfDNA collected in Sarstedt S-Monovette® cfDNA Exact tubes should be stored at **room temperature** until shipping. For best results, ship the samples to LIQOMICS as soon as possible after collection, maintaining room temperature conditions. If immediate shipping is not possible, aim to ship the samples within **48 hours** to maintain sample integrity even if the transport takes longer than usual.
Important: Do not freeze the samples!

2. Packaging and shipping (Illustration 1)
- Place the cfDNA tube in the provided **protective container** and securely **close** it with the supplied lid. Place the sealed container into the cardboard box.
- Include the **completed and signed sample submission sheet**, confirming that the patient has been informed about the test.
  Important: Samples without a completed submission sheet cannot be processed by LIQOMICS.
- Place the cfDNA tube, along with the submission sheet, inside the **provided cardboard box** or other secure packaging.
- Seal the box properly and label it with the supplied **return label**

If you encounter any issues with sample packaging or shipment, feel free to contact us via our webpage, e-mail or phone (see Chapter 11).



Ilustration 1: Sample shipping instructions
Place the completed and signed Sample Submission Form and cfDNA tube in the protective container into the cardboard box provided. Seal the box. Use the provided label to return the package to LIQOMICS. Ship the samples within 48 hours at room temperature!

9 Test results

9.1 How to receive results

An automated report is generated and then technically and medically validated by LIQOMICS. This report is sent to the requesting physician within 2-4 weeks after receipt of the sample, either by fax or per e-mail as password-protected PDF.

9.2 How to communicate results to the patient

The results are communicated to the requesting physician. LIQOMICS does not directly inform the patient about the results. The requesting physician is responsible for complying with all local rules and regulations regarding the communication of genetic test results to the patient. In rare cases, genetic findings unrelated to a performed **Vista** family test may be made during the analysis of the test results. LIQOMICS will inform the requesting physician of any incidental genetic findings, if requested on the submission form by checking the appropriate box. The requesting physician is then responsible for communicating these incidental findings to the patient, in accordance with any local rules and regulations regarding genetic testing.

10 Literature

[1] Validation Report Version 1.0, data on file

[2] SERIAL MONITORING OF CIRCULATING TUMOR DNA WITH AN ULTRASENSITIVE ASSAY FACILITATES OUTCOME PREDICTION IN RELAPSED/REFRACTORY LARGE B-CELL LYMPHOMA TREATED WITH MODERN AGENTS, EHA Library. Schleifenbaum J. 06/13/2024; 419348; P1261 https://library.ehaweb.org/eha/2024/eha2024-congress/419348/julia.k.schleifenbaum.serial.monitoring.of.circulating.tumor.dna.with.an.html

[3] 4355 Lymphovista HL - a Validated Assay for Genotyping and MRD Assessment in Hodgkin Lymphoma, ASH, Annual Meeting & Exposition https://ash.confex.com/ash/2024/webprogram/Paper205034.html

[4] 9.1-LIQ-REC_V2.0_Technical Documentation (in-house IVDs)_VistaFamily of combined ctDNA genotyping and MRD tests.pdf

11 Contact for questions and complaints

If you have any troubles, questions or complaints, you can always contact us before or during the test via our our web contact form[1], by phone, or by e-mail, and we will ensure your concerns are addressed promptly and thoroughly.

Liqomics GmbH
Neurather Ring 1
51063 Köln

+49 221 69 05 65 97
diagnostics@liqomics.com

Mo - Fr 8:00 - 15:00

1 https://liqomics.com/en/contact/