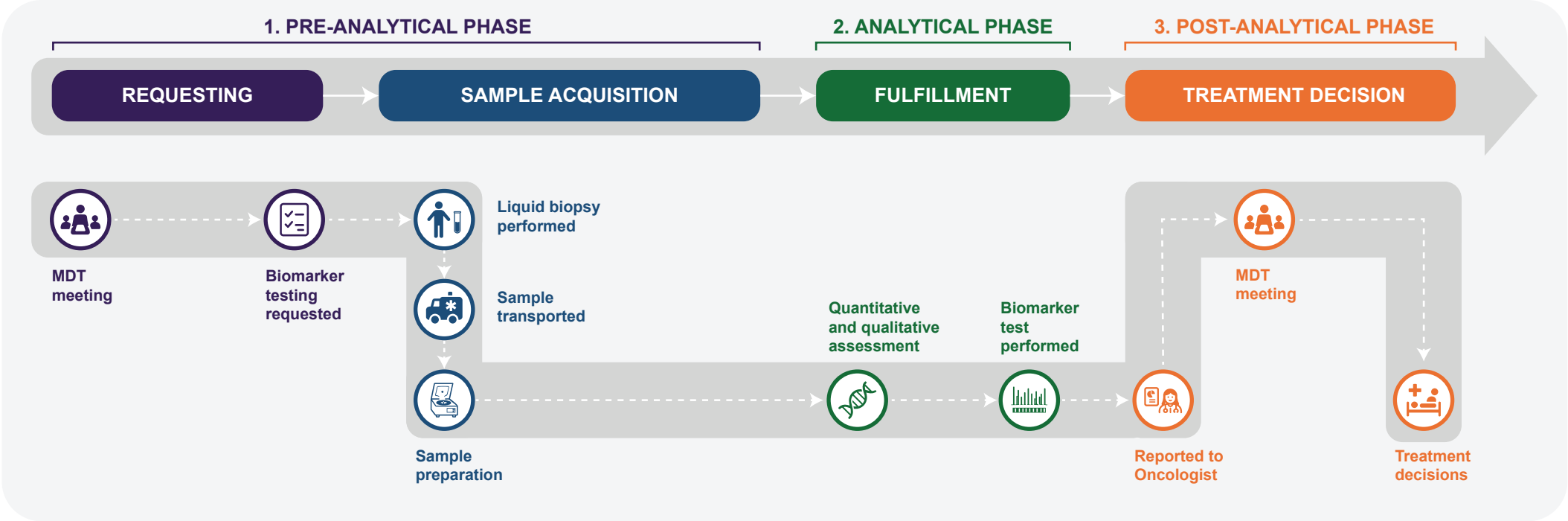
FIELD OF APPLICATION

Standardized procedure for liquid biopsy (LBx) management, structured across pre-analytical, analytical, and post-analytical phases, and based on established best practices to optimize the detection of gene variants associated with response to targeted molecular therapies.

Liquid biopsy represents a minimally invasive approach of increasing relevance in precision medicine, as it enables the dynamic identification and monitoring of genomic variants associated with both response to targeted therapies and the emergence of treatment resistance. Its clinical utility, however, is highly dependent on the quality and standardization of the entire workflow, from sample collection to final reporting.

In this context, close and well-coordinated collaboration between pathologists, laboratory professionals, and oncologists is essential to ensure appropriate test selection, optimal sample handling, robust analytical performance, and accurate clinical interpretation of results, ultimately supporting informed therapeutic decision-making.

The key steps of this workflow and the roles of the multidisciplinary team (MDT) are illustrated in Figure 1.



1. PRE-ANALYTICAL PHASE

1.1.Patient identification

- ☐ Use at least two unique identifiers (e.g. name, surname, tax code)
- ☐ Identity verification upon receipt in the laboratory
- ☐ Include a brief clinical summary in the request form (e.g. patient with lung cancer after first-line treatment with an EGFR inhibitor, or patient with breast cancer after first-line treatment with a CDK4/6 inhibitor plus an aromatase inhibitor)
- ☐ Obtain informed consent

1.2. Sample collection

- ☐ Peripheral blood collected via venipuncture (4–10 mL per tube) — 10–20 mL total volume recommended
- ☐ Immediately after collection, tubes should be gently inverted 8–10 times to ensure proper mixing with the stabilizing reagent
  - ☐ Tubes with stabilizers for ctDNA/ctRNA are preferred (e.g. Streck Cell-Free BCT or PAXgene Blood Tubes)
- ☐ Alternatively, if EDTA tubes are used, perform plasma separation within 2 hours of collection, as described in section 2.1

1.3. Transport and storage

- ☐ Controlled temperature: 18–25 °C
- ☐ Transport within 24–48 hours (maximum 72 hours)
- ☐ Maintain tubes upright during transport at 18–25 °C; avoid agitation, extreme temperatures, direct sunlight, refrigeration, and do not freeze whole blood before plasma separation
- ☐ Mandatory documentation included: patient data, date and time of collection, tube type
- ☐ Non-compliant samples must be reported and managed according to quality procedures.

1.4. Receipt in the laboratory

- ☐ Check tube integrity and verify data consistency
- ☐ Registration in the LIMS
- ☐ Non-eligible samples: suspension and notification

2. ANALYTICAL PHASE

2.1. Plasma isolation

- ☐ Primary centrifugation: 1600–2300 g, 10 minutes, 4 °C
- ☐ Second centrifugation for purification using the same parameters as above
- ☐ Plasma separation avoiding contamination from other samples
- ☐ Preparation of 1.5–2 mL aliquots
- ☐ Immediate extraction or storage under appropriate conditions, as described in section 2.2

2.2. Storage

- ☐ Preferred: –80 °C for long-term
- ☐ Alternative: –20 °C for a maximum of 1 month
- ☐ Avoid repeated freeze–thaw cycles
- ☐ For storage periods longer than one month up to three years, –80°C is recommended. For storage within one month, –20°C can be used, assuming the sample is considered to be in a working context and under active processing.

2.3. Nucleic acids extraction and quality control

- ☐ Extraction from at least 2-4 mL of plasma
- ☐ Automated methods are preferred due to reduced hands-on time, lower risk of contamination, and improved reproducibility
- ☐ Quantification via fluorimetry and real-time PCR

2.4 Molecular analysis

Main technologies available for ctDNA analysis:

- ☐ Digital PCR
- ☐ Next-Generation Sequencing (broad reference range and identification of new variants)
- ☐ Real-time PCR / Allele-Specific PCR

3. POST-ANALYTICAL PHASE

3.1. Result validation – NGS bioinformatics parameters

- ☐ Mapped reads percentage >95%
- ☐ Mean coverage ≥5000x
- ☐ Coverage uniformity >90%

3.2. Variant detection and interpretation

- ☐ Allelic frequency, considering LOD (limit of detection)
- ☐ Use of updated clinical databases (e.g. Varsome, Franklin by Qiagen, OncoKB) for classification: pathogenic, benign, VUS
- ☐ Application of clinical evidence scales (ESMO ESCAT) to provide therapeutic recommendations

3.3. Report

Must include:

- ☐ Header with clinical data and patient/sample identifiers, and analytical method used
- ☐ List of detected mutations with corresponding clinical significance and level of evidence
- ☐ Any therapeutic recommendations
- ☐ Limitations (DNA quality and quantity, method sensitivity/LOD, possible false negatives)
- ☐ Signature and contact details

4. STORAGE OF MATERIAL

4.1. Data and file storage

- ☐ Pseudonymized data
- ☐ Storage in standard formats (PDF, VCF, BAM, FASTQ)
- ☐ Retention:
  - VCF ≥20 years
  - BAM/FASTQ ≥2 years

4.2. Regulations

- ☐ GDPR (Legislative Decree 101/2018)
- ☐ Ministry of Health guidelines
- ☐ SIGU
- ☐ ISO 20387

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BLOOD COLLECTION PROCEDURE AND TUBES



- Hemolysis must be prevented
- Use of butterfly needles is recommended
- Mix by gentle inversion
- 10 mL for genotyping
- Up to 20mL for MRD and localized disease



EDTA

- Fast processing within 2-4h

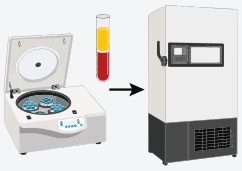


STABILIZATION TUBES

- Samples storage up to 14 days at RT
- Transportation at RT
- Glass BCTs in safety bags

CENTRIFUGATION AND PLASMA STORAGE

- Two-step centrifugation protocol
- First low-speed step and second with higher velocity
- Light break in the first centrifugation step



- Storage at -80 °C
- Plasma aliquots from 300uL to 2mL are preferred
- Freeze-thaw cycles should be avoided

cfDNA ISOLATION



- Silica-based membrane columns
- Magnetic beads
- Commercial kits available (with or without automation)

QUANTIFICATION AND QUALIFICATION



- Fluorescence-based assays
- PCR-based tools
- Tumor fraction quantification helps defining truly negative results

ANALYSIS

REFERENCES (DOIs):

https://doi.org/10.1016/j.bdq.2019.100087
https://doi.org/10.1016/j.ctrv.2023.102595
https://doi.org/10.1016/j.critrevonc.2024.104427
https://doi.org/10.1016/j.annonc.2022.05.520
https://doi.org/10.1016/j.jlb.2025.100314

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Disclaimer

The PCC is a non-profit organization dedicated to driving global access to comprehensive biomarker testing for all patients with cancer. The PCC is composed of and funded by: AstraZeneca, Bristol Myers Squibb, Johnson & Johnson, and Lilly at the time of writing. PCC-337, Date of preparation: November 2026.