



## International Journal of Cancer Research and Therapeutic Studies

Journal homepage: <https://trpub.org/journal.php?id=XTjoTjoA2a>

TRPub

IJCRTS

International Journal of  
Cancer Research and  
Therapeutic Studies

Vol. 1 Issue 1 2026

Methodology Article

Open Access

# A Liquid Biopsy Workflow for Monitoring Minimal Residual Disease in Colorectal Cancer

Jorge Radovic<sup>1\*</sup>, Joaquin Rao<sup>2</sup>, Hirut Kenzhebekov<sup>3</sup>

<sup>1</sup>Department of Medical Sciences, Bharathapuzha Center for Medical Sciences, Ireland

<sup>2</sup>Department of Medical Sciences, Loire Institute for Advanced Medical Sciences, Barbados

<sup>3</sup>Department of Medical Sciences, Tagus School of Medical Sciences, Vietnam

### Article Info

Volume 1, Issue 1, 2026

doi: [10.62715/ijcrts.2026.v1.i1.pp15-20](https://doi.org/10.62715/ijcrts.2026.v1.i1.pp15-20)

Received: 22 September 2025

Accepted: 09 November 2025

Published: 10 January 2026

### Abstract

After resection of a colorectal cancer with curative intent, the clinical problem is to know which patients still harbor disease that imaging cannot show. Fragments of tumor DNA circulating in plasma offer a direct sign of such residual disease, and observational cohorts and one randomized trial have established that their detection after surgery identifies patients at high risk of recurrence. Laboratories and clinical teams that wish to set up testing face a long chain of decisions in which a weakness at any link can invalidate the result. This article proposes a workflow in seven steps: profiling of the tumor and matched normal tissue, choice between a tumor-informed and a tumor-agnostic assay, blood collection and processing, extraction and quantification of cell-free DNA, sequencing with molecular error suppression, calling of the result against rules fixed in advance, and reporting within a schedule of serial sampling. For each step the purpose, the main sources of error and the record to be kept are described. The workflow is assembled from published methods and consensus statements. It is a proposal that has not been applied or validated here, and the article sets out what a laboratory would need to demonstrate before using it to inform care.

**Keywords:** *Circulating tumor DNA, Minimal residual disease, Colorectal cancer, Liquid biopsy, Pre-analytical variables, Surveillance*

© 2026 Jorge Radovic et al. This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## 1. Introduction

Surgery cures many patients with stage II and III colorectal cancer, and adjuvant chemotherapy cures some of the remainder. The decision to give that chemotherapy has rested on pathological stage and a short list of risk features, which sort patients into groups without identifying the individuals who have residual tumor cells. The consequence is treatment of many who were cured by the operation and undertreatment of some who were not.

Tumors shed fragments of DNA into the bloodstream, and these can be recognized by the somatic mutations they carry. Diehl et al. (2008) showed in colorectal cancer that the level of mutant DNA in plasma falls quickly after complete resection and that its persistence precedes relapse. Later work with

\* Corresponding author: Jorge Radovic, Department of Medical Sciences, Bharathapuzha Center for Medical Sciences, Ireland. E-mail: [jorge.radovic@bharathapuzha.edu](mailto:jorge.radovic@bharathapuzha.edu)

© 2026 Jorge Radovic et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

more sensitive assays confirmed that detection of circulating tumor DNA (ctDNA) in the weeks after surgery marks a group with a very high risk of recurrence (Tie et al., 2016; Reinert et al., 2019). The term minimal residual disease, borrowed from hematology, is now applied to this state, and some authors prefer molecular residual disease.

This article is written for laboratories and multidisciplinary teams planning to introduce such testing. It sets out a complete workflow from tissue to report, with the reasoning behind each step. No samples were analyzed for it. The procedure is proposed on the basis of the published literature and has not been validated by the author; the final sections state what validation would involve.

2. Background

Three bodies of knowledge inform the design: the biology that makes the measurement difficult, the two families of assay available, and the clinical evidence that defines what the test is for.

2.1. Why the measurement is difficult

Plasma from a patient without bulky disease contains only small quantities of cell-free DNA (cfDNA), nearly all of it released by blood cells. In localized cancers the tumor-derived share is far smaller than in metastatic disease and is undetectable in a proportion of patients (Bettegowda et al., 2014). After resection it can fall well below one mutant molecule in a thousand. At such levels two limits apply. One is sampling: a tube of blood holds a finite number of genome copies, and a mutation absent from the tube cannot be found by any chemistry. The other is error, since polymerase and sequencer mistakes occur at rates comparable to the signal sought (Wan et al., 2017).

A third difficulty is biological. With age, blood stem cells acquire somatic mutations and expand as clones, a process termed clonal hematopoiesis (Jaiswal et al., 2014). Deep sequencing of plasma has shown that a large share of the variants found in cfDNA originate from these clones and not from tumor (Razavi et al., 2019). Unless they are filtered, they produce false positive results.

2.2. Two families of assay

Assays for residual disease are of two kinds, compared in Table 1. A tumor-informed assay begins by sequencing the resected tumor, selects a set of that patient's own somatic variants and builds a personalized panel to track them in plasma, an approach used in the studies of Reinert et al. (2019) and Tie et al. (2016). A tumor-agnostic, or plasma-only, assay applies one fixed panel to every patient and commonly adds methylation patterns characteristic of colorectal cancer to raise sensitivity (Parikh et al., 2021). Neither is superior on every count, and the choice depends on tissue availability, turnaround and laboratory capacity.

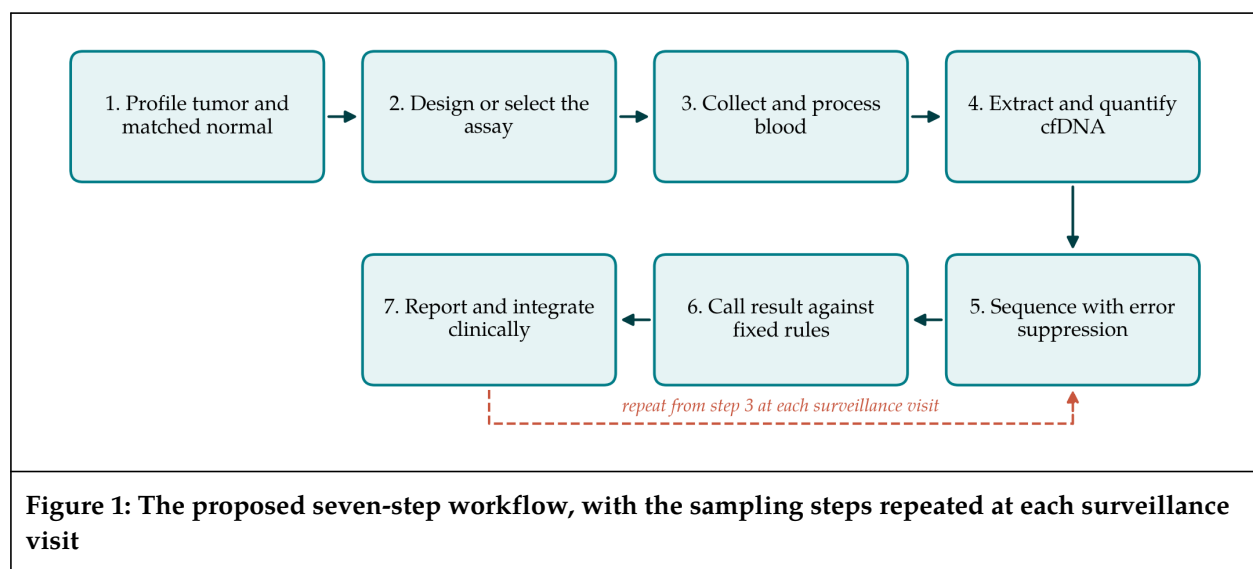
| Table 1: Comparison of tumor-informed and tumor-agnostic approaches to detecting residual disease in plasma |                                                   |                                                    |
|-------------------------------------------------------------------------------------------------------------|---------------------------------------------------|----------------------------------------------------|
| Feature                                                                                                     | Tumor-informed assay                              | Tumor-agnostic assay                               |
| Starting material                                                                                           | Tumor tissue, matched normal and plasma           | Plasma, ideally with white cells                   |
| What is tracked                                                                                             | A set of the patient's own somatic variants       | Fixed gene panel, often with methylation markers   |
| Time to first result                                                                                        | Longer, because the panel is built per patient    | Shorter, no bespoke design                         |
| Clonal hematopoiesis                                                                                        | Largely excluded by design                        | Must be filtered by white cell sequencing or rules |
| Dependence on tissue                                                                                        | Fails if tissue is scant or of poor quality       | None                                               |
| Tumor evolution                                                                                             | New clones without tracked variants can be missed | May detect changes not present in the primary      |

### 2.3. What the clinical evidence supports

Prospective cohorts agree that postoperative ctDNA is a strong prognostic marker (Tie et al., 2016; Reinert et al., 2019; Henriksen et al., 2022). A large Japanese observational study added that patients with detectable ctDNA after surgery fared better with adjuvant chemotherapy than without it, while no such difference was apparent in those who tested negative (Kotani et al., 2023). The randomized DYNAMIC trial in stage II colon cancer showed that a ctDNA-guided strategy reduced the use of adjuvant chemotherapy without worsening recurrence-free survival (Tie et al., 2022). Guidance has nonetheless remained cautious. A joint review by two American professional societies found the evidence for clinical utility in early-stage disease insufficient at the time (Merker et al., 2018), and a European working group later judged that residual disease assays, although clinically valid, should not yet direct routine treatment (Pascual et al., 2022). The workflow below should be read in that light.

### 3. The Proposed Workflow

The workflow has seven steps, shown in order in Figure 1. Steps 1 and 2 are performed once for each patient. Steps 3 to 7 are repeated at every blood draw.



#### 3.1. Step 1: Profile the tumor and matched normal

A pathologist selects a block from the resection specimen with adequate tumor cellularity, and DNA is extracted from it and from a normal sample, usually the buffy coat of a blood draw. Sequencing both allows germline variants and clonal hematopoiesis to be subtracted, leaving the somatic variants of the tumor. Variants present in most tumor cells are preferred as targets, because a subclonal variant may be absent from the cells that seed a recurrence. The record should state the block used, the estimated tumor fraction, the sequencing depth and the variants chosen.

#### 3.2. Step 2: Design or select the assay

If a tumor-informed approach is taken, a panel covering the selected variants is designed and its performance checked on the patient's own tumor and normal DNA. Tracking several variants at once raises the chance that at least one mutant fragment is present in a given tube. If a fixed panel is used, this step reduces to confirming that the panel is suitable for colorectal cancer and that a method for excluding clonal hematopoiesis is in place. In either case the assay and its version are fixed before the first plasma sample is tested.

#### 3.3. Step 3: Collect and process blood

Errors made before the sample reaches the laboratory are among the most frequent and the least recoverable. Plasma is used in preference to serum, because clotting releases DNA from white cells and

dilutes the tumor fraction. Blood drawn into EDTA tubes must be separated within a few hours; tubes containing a cell-stabilizing preservative allow longer transport at ambient temperature. Two centrifugation steps remove cells and debris, and plasma is stored frozen at  $-80^{\circ}\text{C}$  until extraction (Merker et al., 2018). The time of draw, tube type, time to processing and plasma volume are recorded for every sample.

Timing relative to treatment matters as much as handling. Surgery injures tissue and releases normal cfDNA for some weeks, which can mask a small tumor signal, and chemotherapy may suppress shedding without eliminating disease. The first postoperative sample is therefore best taken about a month after resection and before adjuvant treatment begins, as in the DYNAMIC trial (Tie et al., 2022), with the schedule agreed in advance (Dasari et al., 2020).

### **3.4. Step 4: Extract and quantify cell-free DNA**

cfDNA is extracted with a method validated for short fragments, and the yield is measured. The quantity matters for interpretation and is more than a quality check. The number of genome copies entered into the assay sets the lowest tumor fraction that could have been detected in that sample, and a negative result from a low-input sample is weaker than one from a generous input. A fragment size profile helps to reveal contamination by high-molecular-weight DNA from lysed white cells.

### **3.5. Step 5: Sequence with error suppression**

Detection at very low allele fractions depends on separating true mutations from artifacts. The standard solution is to tag each original DNA molecule with a unique molecular identifier before amplification, so that reads descending from one molecule can be grouped and a consensus taken. Errors that appear in only some reads of a family are then discarded (Kinde et al., 2011). Hybrid-capture methods combine such barcoding with statistical models of background error at each position (Newman et al., 2014). Whatever the chemistry, every run should include a negative control and a positive control at a known low allele fraction.

### **3.6. Step 6: Call the result against fixed rules**

The criteria for a positive sample are written down before clinical testing starts and are not adjusted case by case. For a tumor-informed assay a typical rule requires that a minimum number of tracked variants be detected above the background established in healthy donors. For a fixed panel, any variant also found in the patient's white cells is removed as clonal hematopoiesis (Razavi et al., 2019). The output is categorical, detected or not detected, accompanied by a quantitative estimate and by the input amount from step 4.

### **3.7. Step 7: Report and integrate into follow-up**

The report states the assay type, the result, the quantity of cfDNA analyzed and a plain statement of what a negative result does and does not exclude. Results are discussed alongside stage, pathology, carcinoembryonic antigen and imaging. A single negative sample soon after surgery leaves a real chance of later relapse, and serial testing during surveillance raises sensitivity for recurrence (Reinert et al., 2019). The interval between draws and the action to follow a positive result should be set by the team beforehand, since a result with no agreed consequence causes anxiety without benefit.

## **4. Fitting the Workflow to a Service**

Few hospitals will perform every step themselves. A common arrangement is local collection and plasma separation, with sequencing in a central or commercial laboratory. In that case steps 3 and 7 are where the local team controls quality, and written agreement is needed on tube type, shipping conditions and the content of the report. Turnaround is a practical constraint: adjuvant chemotherapy is normally started within a couple of months of surgery, so a bespoke assay that delivers its first result late may arrive after the decision it was meant to inform. Cost, and the absence of reimbursement in many systems, will decide for some services whether testing is offered at all or confined to research protocols, which is the setting current European guidance favors (Pascual et al., 2022).

## 5. Limitations and What Remains to Be Shown

The workflow is a synthesis of published practice and has not been run or validated for this article. A laboratory adopting it would first need to establish analytical performance: the limit of detection at defined inputs, specificity in plasma from healthy donors, and reproducibility between runs and operators. Clinical performance against recurrence would then need to be measured in its own population and reported according to the STARD statement for diagnostic accuracy studies (Bossuyt et al., 2015).

Some limits are inherent in the method. Tumors differ in how much DNA they shed, and recurrences confined to the lung or peritoneum are reported to be detected less readily than liver metastases. A negative test cannot prove cure. Whether acting on a positive result by intensifying or prolonging chemotherapy improves survival is the subject of ongoing randomized trials and is not yet known (Dasari et al., 2020). Until those trials report, the test is best regarded as a well-validated prognostic marker whose use in treatment decisions belongs within trials or carefully governed local protocols.

## 6. Conclusion

Monitoring residual disease by ctDNA after colorectal cancer surgery is technically demanding and feasible. The seven steps proposed here link tissue profiling, assay choice, careful blood handling, quantified extraction, error-suppressed sequencing, pre-specified calling and structured reporting, and attach a record to each. The approach rests on methods others have published, and its performance in any given laboratory remains to be demonstrated. Used with that understanding, it offers a way to prepare for a test that may soon change how adjuvant treatment is chosen.

## Conflict of interest

To be declared by the author(s) before publication.

## Funding

To be declared by the author(s) before publication.

## References

- Bettegowda, C., Sausen, M., Leary, R.J. et al. (2014). [Detection of circulating tumor DNA in early- and late-stage human malignancies](#). *Science Translational Medicine*, 6(224): 224ra24. <https://doi.org/10.1126/scitranslmed.3007094>
- Bossuyt, P.M., Reitsma, J.B., Bruns, D.E. et al. (2015). [STARD 2015: An updated list of essential items for reporting diagnostic accuracy studies](#). *BMJ*, 351: h5527. <https://doi.org/10.1136/bmj.h5527>
- Dasari, A., Morris, V.K., Allegra, C.J. et al. (2020). [ctDNA applications and integration in colorectal cancer: An NCI Colon and Rectal-Anal Task Forces whitepaper](#). *Nature Reviews Clinical Oncology*, 17(12): 757-770. <https://doi.org/10.1038/s41571-020-0392-0>
- Diehl, F., Schmidt, K., Choti, M.A. et al. (2008). [Circulating mutant DNA to assess tumor dynamics](#). *Nature Medicine*, 14(9): 985-990. <https://doi.org/10.1038/nm.1789>
- Henriksen, T.V., Tarazona, N., Frydendahl, A. et al. (2022). [Circulating tumor DNA in stage III colorectal cancer, beyond minimal residual disease detection, toward assessment of adjuvant therapy efficacy and clinical behavior of recurrences](#). *Clinical Cancer Research*, 28(3): 507-517.
- Jaiswal, S., Fontanillas, P., Flannick, J. et al. (2014). [Age-related clonal hematopoiesis associated with adverse outcomes](#). *New England Journal of Medicine*, 371(26): 2488-2498. <https://doi.org/10.1056/NEJMoa1408617>
- Kinde, I., Wu, J., Papadopoulos, N., Kinzler, K.W. and Vogelstein, B. (2011). [Detection and quantification of rare mutations with massively parallel sequencing](#). *Proceedings of the National Academy of Sciences of the United States of America*, 108(23): 9530-9535. <https://doi.org/10.1073/pnas.1105422108>
- Kotani, D., Oki, E., Nakamura, Y. et al. (2023). [Molecular residual disease and efficacy of adjuvant](#)

- chemotherapy in patients with colorectal cancer. *Nature Medicine*, 29(1): 127-134. <https://doi.org/10.1038/s41591-022-02115-4>
- Merker, J.D., Oxnard, G.R., Compton, C. et al. (2018). Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. *Journal of Clinical Oncology*, 36(16): 1631-1641. <https://doi.org/10.1200/JCO.2017.76.8671>
- Newman, A.M., Bratman, S.V., To, J. et al. (2014). An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nature Medicine*, 20(5): 548-554. <https://doi.org/10.1038/nm.3519>
- Parikh, A.R., Van Seventer, E.E., Siravegna, G. et al. (2021). Minimal residual disease detection using a plasma-only circulating tumor DNA assay in patients with colorectal cancer. *Clinical Cancer Research*, 27(20): 5586-5594. <https://doi.org/10.1158/1078-0432.CCR-21-0410>
- Pascual, J., Attard, G., Bidard, F.-C. et al. (2022). ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: A report from the ESMO Precision Medicine Working Group. *Annals of Oncology*, 33(8): 750-768. <https://doi.org/10.1016/j.annonc.2022.05.520>
- Razavi, P., Li, B.T., Brown, D.N. et al. (2019). High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. *Nature Medicine*, 25(12): 1928-1937. <https://doi.org/10.1038/s41591-019-0652-7>
- Reinert, T., Henriksen, T.V., Christensen, E. et al. (2019). Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncology*, 5(8): 1124-1131. <https://doi.org/10.1001/jamaoncol.2019.0528>
- Tie, J., Cohen, J.D., Lahouel, K. et al. (2022). Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. *New England Journal of Medicine*, 386(24): 2261-2272. <https://doi.org/10.1056/NEJMoa2200075>
- Tie, J., Wang, Y., Tomasetti, C. et al. (2016). Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. *Science Translational Medicine*, 8(346): 346ra92. <https://doi.org/10.1126/scitranslmed.aaf6219>
- Wan, J.C.M., Massie, C., Garcia-Corbacho, J. et al. (2017). Liquid biopsies come of age: Towards implementation of circulating tumour DNA. *Nature Reviews Cancer*, 17(4): 223-238. <https://doi.org/10.1038/nrc.2017.7>