# Extraction of Multiple Analytes in Liquid Biopsy May Improve the Diagnosis of Breast Cancer

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## INTRODUCTION

Since a single biomarker is inefficient in accurately identifying most cancers, integrated liquid biopsy using multiple biomarkers might be a promising method to facilitate early detection and treatment of cancer. This study demonstrates an optimized extraction method for cfTNA (Cell-Free Total Nucleic Acid) and Circulating Tumor Cells (CTC) from a single blood sample.

The nRichDX cfTNA and CTC Isolation kits can simultaneously extract high-quality cfDNA, cfRNA, and CTCs with high specificity from a single blood sample.

## MATERIALS & METHODS

Human whole blood was collected in K2EDTA tubes. The whole blood was spiked with 100 MCF-7 cells and processed at 800xg for 10 minutes to separate plasma from whole blood. Plasma was carefully removed and then centrifuged at 16,000xg for 10 minutes at 4˚ C to obtain cell-free plasma.

MCF-7 cells were extracted from six 10mL samples of whole blood using the nRichDX Revolution CTC Isolation Kit. The recovery of the extracted MCF-7 cells was measured by immunocytochemistry (ICC) using primary antibodies EpCAM (Ab71916), CD45 (Ab30470), and CK8 (Ab9023). The slides were imaged using an LSM900 microscope.

cfRNA and cfDNA were extracted from six 5mL cf-plasma samples using the nRichDX Revolution Total Nucleic Acid Kit. All samples were spiked with a cfDNA standard and RNA standard containing the PIK3CA E545K mutation at a concentration of 20ng/mL. All six samples were eluted in 50µL. The quality of cfDNA and cfRNA was determined using the Agilent High Sensitivity RNA ScreenTape®. The recovery of the PIK3CA E545K mutation from plasma samples was measured using the ThermoFisher TaqMan Assay (Cat No. 4465804).

## RESULTS

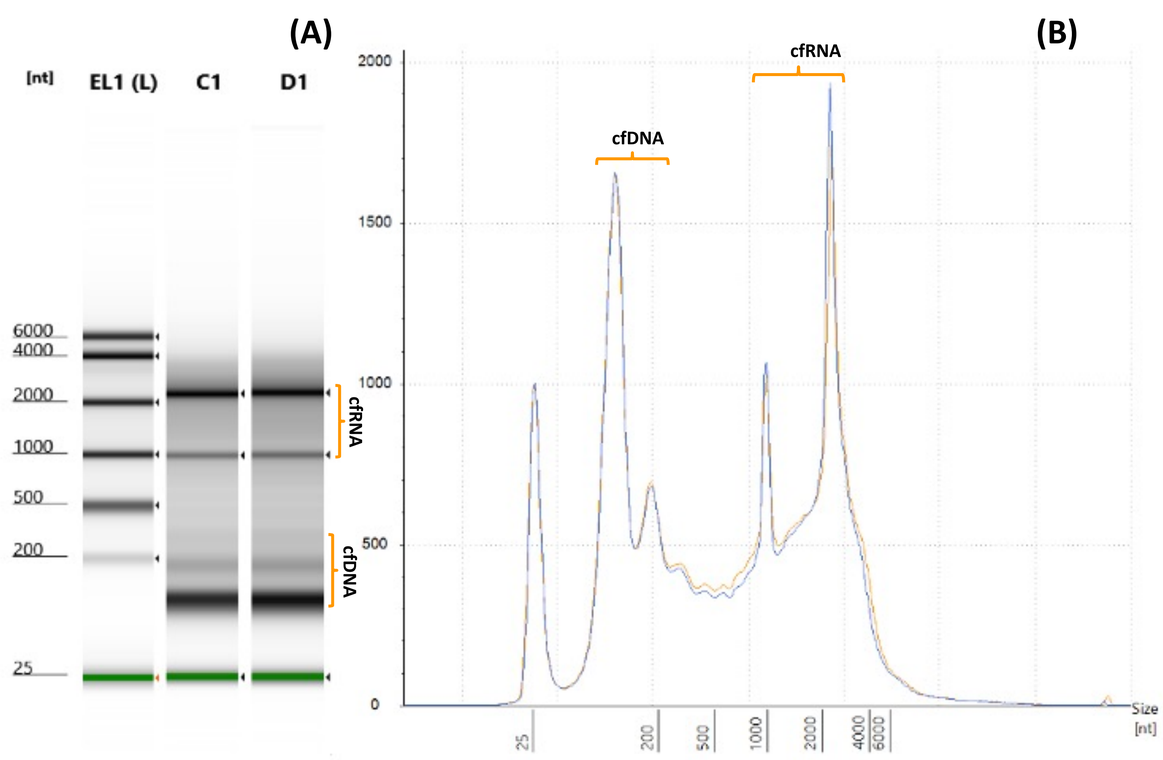


**Figure 1. (A) Gel electrophoresis of the extracted nucleic acids size (bp).** Column EL1 is the electronic ladder, C1 and D1 represent the extracted nucleic acids by the nRichDX Total Nucleic Acid kit. cfDNA and cfRNA peaks were labeled on the gel.
**(B) Electropherogram of Cell-free DNA and Cell-Free RNA in plasma.** cfDNA and cfRNA extracted from plasma were analyzed using Agilent High Sensitivity RNA ScreenTape. The tracings indicate a high intensity of cfDNA and cfRNA extracted simultaneously.

| CTC Percent Recovery | |
|---|---|
| **Sample** | **CTC Count (cells)** |
| 1 | 92 |
| 2 | 81 |
| 3 | 76 |
| 4 | 93 |
| 5 | 87 |
| 6 | 96 |
| Mean CTC Percent Recovery (%) | 88% |

**Table 2.** CTC recovery of 100 EpCAM positive MCF-7 cells spiked into six 10mL whole blood samples using the nRichDX CTC Isolation Kit.

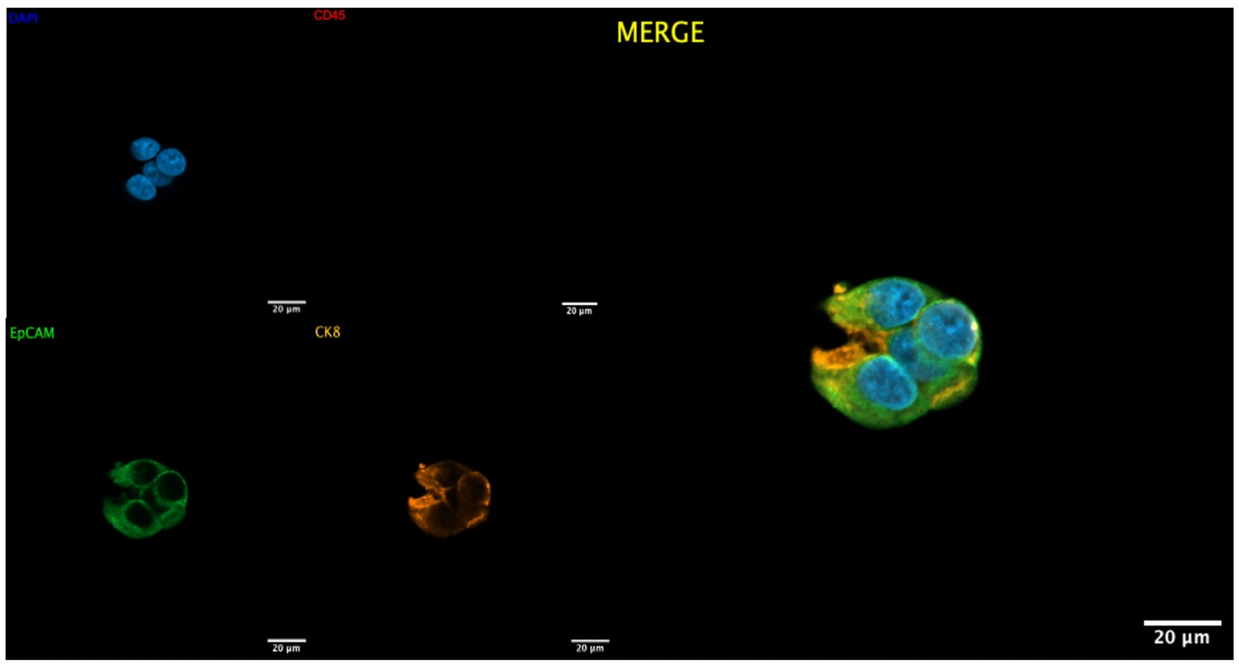


**Figure 2. Representative immunofluorescent microscopy images of MCF-7 cells extracted using the nRichDX CTC Isolation Kit**. MCF7 cells extracted with the nRichDX system were imaged using Immunofluorescent microscopy which is positive for DAPI (blue), EpCAM (green), CK8 (orange), and negative for CK45 (red).

| PIK3CA E545K RNA RT-PCR | | |
|---|---|---|
| **Sample** | **Ct** | **Mean Ct** |
| 1 | 30.8 | 31.1 |
| 2 | 30.8 | |
| 3 | 31.4 | |
| 4 | 31.2 | |
| 5 | 31.0 | |
| 6 | 31.1 | |
| RNA Positive Control | 33.2 | 33.0 |
| | 32.9 | |
| DNA Positive Control | 30.5 | 30.3 |
| | 30.2 | |

| PIK3CA E545K DNA PCR | | |
|---|---|---|
| **Sample** | **Ct** | **Mean Ct** |
| 1 | 34.5 | 34.4 |
| 2 | 34.3 | |
| 3 | 34.3 | |
| 4 | 34.1 | |
| 5 | 34.8 | |
| 6 | 34.6 | |
| DNA Positive Control | 33.7 | 33.7 |
| | 33.6 | |

**Table 1. Quantification of PIK3CA mutation detection assay for RNA and DNA.** Ct's obtained via RT-PCR and PCR demonstrated the recovery of cfDNA and cfRNA containing the PIK3CA E545K mutation.

## CONCLUSION

qPCR mutation detection assay demonstrated high recovery of the PIK3CA E545K mutation spiked into the sample as free RNA and DNA. TapeStation tracings showed prominent monomer, dimer, and trimer peaks for cfDNA and pronounced ribosomal tracing peaks for cfRNA. Immunocytochemistry (ICC) showed recovery of 88% of the MCF-7 cells spiked in all samples. The circulating tumor cells (CTC) recovery was demonstrated by positive fluorescent staining of EpCAM, CK8, and negative fluorescent staining of CD45.

This study demonstrates an optimized extraction method for cfTNA (Cell-free Total Nucleic Acid) and CTC from a single blood sample. By integrating a diverse array of biomarkers, this liquid biopsy approach offers a comprehensive and dynamic assessment of the disease. These biomarkers encompass various genetic, epigenetic, and proteomic alterations, providing a holistic view of the tumor's characteristics and behavior.